Vol. 6
1961

Archives of Oral Biology

Volume 6, 1961

Proceedings of the 8th Congress of the
European Organization for Research
on Fluorine and Dental Caries Prevention

London, July 1961

Edited by
ORCA

PERGAMON PRESS LTD.
Oxford London New York Paris

EDITORIAL BOARD

Prof. Dr. med. H. D. CREMER, Institut für Ernährungswissenschaft der Justus Liebig-Universität Giessen, Wilhelmstrasse 20, Giessen, Germany.

Dr. R. M. FRANK, Institut Dentaire, Faculté de Médecine, Strasbourg, France.

Dr. R. C. GREULICH, Department of Anatomy, University of California Medical Center, Los Angeles 24, California.

Mr. IVOR R. H. KRAMER, Institute of Dental Surgery, Eastman Dental Hospital, Gray's Inn Road, London W.C.1.

Dr. J. B. MACDONALD, The Forsyth Dental Infirmary, 140 The Fenway, Boston 15, Mass., U.S.A.

Professor A. E. W. MILES, Department of Dental Histology and Pathology, London Hospital Medical College, Turner Street, London E.1.

Dr. M. V. STACK, University of Bristol Dental School, Lower Maudlin Street, Bristol 1.

HONORARY EDITORIAL ADVISORY BOARD

P. ADLER (Debrecen, Hungary)
A. B. P. AMIES (Melbourne)
F. A. ARNOLD, Jr.* (Bethesda, Md.)
O. BACKER-DIRKS (Utrecht)
K. A. BISSET (Birmingham, England)
F. BRAMSTEDT (Hamburg)
F. A. CARRANZA, Jr. (Buenos Aires)
D. H. COPP (Vancouver)
H. DALLEMAGNE (Liège)
G. N. DAVIES (Dunedin, New Zealand)
D. DZIEWIATKOWSKI (New York)
Y. ERICSSON (Stockholm)
R. O. GREEP (Boston)
R. L. HARTLES (Liverpool)
D. HATTYASY (Szeged, Hungary)
J. W. HEIN (New Jersey)
A. J. HELD (Geneva)
E. E. HUNT (Boston)
J. T. IRVING (Boston)
G. N. JENKINS (Newcastle upon Tyne)
B. S. KRAUS (Seattle)
C. P. LEBLOND (Montreal)
B. LILIENTHAL (Melbourne)
S. MATSUMIYA (Tokyo)
M. L. MOSS (New York)
H. R. MÜHLEMANN (Zurich)
G. NIKIFORUK (Toronto)
P. O. PEDERSEN (Copenhagen)
R. W. PHILLIPS (Indianapolis)
W. PIGMAN (New York)
H. B. G. ROBINSON (Kansas City)
T. ROSEBURY (St. Louis)
M. A. RUSHTON* (London)
A. L. RUSSEL (Bethesda, Md.)
I. SCHOUR (Chicago)
D. B. SCOTT (Bethesda, Md.)
F. R. SHROFF (Dunedin, New Zealand)
R. F. SOGNNAES (Boston)
J. STAZ (Johannesburg)
A. SYRRIST (Malmö)
R. J. S. TICKLE (Singapore)
J. F. VOLKER (Birmingham, Alabama)
J. WAERHAUG (Oslo)
J. P. WALSH (Dunedin, New Zealand)
Y. WATANABE (Okayama)
N. B. WILLIAMS (Philadelphia)

* *Joint Chairmen of the Board*

Publishing Offices: Headington Hill Hall, Oxford (Oxford 64881)

Annual subscription (including postage):

(A) For libraries, government establishments and research institutions £14 ($40.00)

(B) For individuals who write directly to the publisher and certify that the journal is for their personal use £5 5s. ($15.00)

Copyright © 1961
Pergamon Press Ltd.

PERGAMON PRESS LTD.

Headington Hill Hall,
Oxford

122 East 55th Street,
New York 22, N.Y.

Vol. 6
1961

EDITORIAL NOTE

The papers in this volume were presented at the ORCA Meeting held in London during July 1961. They have not been submitted to the usual refereeing and no editorial amendments have been made by the Editors of *Archives of Oral Biology*.

CONTENTS OF VOLUME 6

Proceedings of 8th Congress of the European Organization for Research on Fluorine and Dental Caries Prevention

page

K. E. QUENTIN: Fluorbestimmung in fluoridiertem Speisesalz 1
C. P. WALLIS: Measurement of caries progress 7
W. HOFFMANN-AXTHELM: Acht Jahre Kariesstatistik in einem endemischen Fluorgebiet . 12
H. LEONHARDT: Ergebnisse der Fluortablettenzufuhr bei Salzburger Schulkindern im Alter von 6–10 Jahren, 4 Jahre nach Beginn der Aktion 17
GISELA SCHÜTZMANNSKY: Sechs Jahre Kariesprophylaxe durch Fluortablettengaben . . 27
H. J. WESPI: Experiences and problems of fluoridated cooking salt in Switzerland . . 33
W. BÜTTNER: Effects of some trace elements on fluoride retention and dental caries . . 40
A. L. RUSSELL and PEGGY M. HAMILTON: Dental caries in permanent first molars after eight years of fluoridation 50
E. WELANDER: Problems of recording and sampling in clinical investigations of caries . 58
J. MILLER: Relationship of occlusion and oral cleanliness with caries rates . . . 70
D. JACKSON: An epidemiological study of dental caries prevalence in adults . . . 80
O. BACKER DIRKS: Longitudinal dental caries study in children 9–15 years of age . . 94
P. SCHLATTER, T. M. MARTHALER and H. R. MÜHLEMANN: Changes in the depth of artificial marks on tooth surfaces after certain time intervals 109
S. A. LEACH: The effect of various anions on the solubility of enamel and dentine in acid 119
I. PORCSALMY, P. ADLER and Z. NAGY: The storage of molybdenum 125
R. L. HARTLES: Citrate in bone and dentine 130
A. KRÖNCKE: Transaminasen im menschlichen Speichel 134
L. GRIMBERT et G. PIGEAT: Examen des répliques de la surface de l'émail au moyen du microscope à contraste interférentiel (système Nomarski) 139
H. EGGERS-LURA: New investigations into the cariogenic properties of dental plaques . 149
H. TAATZ: Histologische Studien an Nagezähnen des syrischen Goldhamsters . . . 161
H. NEWESELY: Changes in crystal types of low solubility calcium phosphates in the presence of accompanying ions 174
G. HENKEL: Der Fluorgehalt von Trinkwässern aus verschiedenen geologischen Formationen Thüringens 181
H. LINGELBACH: Chemisch-technische Erfahrungen der Trinkwasserfluoridierung in Karl-Marx-Stadt—II. Bericht 193
K. BINDER: Caries frequency and fluorosis in Mallnitz and the valley of Ötz (1·0–1·8 p.p.m. F in the drinking water) 198
D. TOMIĆ: Der Fluoridgehalt montenegrinischer Trinkwässer—II 203
M. JIRÁSKOVÁ: An epidemiologic study of the caries incidence in localities with an optimal content of fluorine in drinking water 209
K. G. KÖNIG: Effects of mastication and particle size of corn and sugar diets on caries-incidence in rats 214
T. NEUBAUER und R. NAUJOKS: Über die Spaltung von Saccharose und Maltose im menschlichen Speichel 221

Vol. 6
1961

B. RANKE: Über den Einfluss von Metallen auf die Glykolyse im menschlichen Speichel 228
E. RANKE und B. RANKE: Über den Sediment- und Proteingehalt des Speichels kariesanfälliger und kariesresistenter Personen 234
G. AHRENS: Beziehungen zwischen dem Phosphatgehalt des Speichels und Karies . . 241
D. HATTYASY, I. SZABÓ and K. TÓTH: Some physico-chemical properties of the carious and non-carious saliva 249
R. DUCKWORTH and R. D. EMSLIE: Fluoridated chewing gum and toothpaste studies with ^{18}F 260
T. M. MARTHALER, ANGELA SCHAIT and H. R. MÜHLEMANN: Dissolution of dental enamel in decalcifying buffers containing different amounts of calcium and phosphorus . 266
R. SÖREMARK and K. SAMSAHL: Gamma-ray spectrometric analysis of elements in normal human enamel 275
F. VON BARTHELD: Membrane phenomena in carious dissolution of the teeth . . . 284
F. BRUDEVOLD, B. H. AMDUR and A. MESSER: Factors involved in remineralization of carious lesions 304
G. N. JENKINS: The biochemistry of enamel in relation to caries-resistance . . . 305
F. A. ARNOLDIR: Recent advances in dental research in the U.S.A. 315
S. FIORENTINI et F. DI BLASI: Altérations rénales et hepatiques chez des rats albinos traités par microdoses de NaF 317

Vol. 6
1961

AUTHOR INDEX

Volume 6 1961

Adler, P. p. 125
Ahrens, G. p. 241
Amdur, B. H. p. 304
Arnold, F. A., Jr. p. 315
Backer Dirks, O. p. 94
Bartheld, F. von p. 284
Binder, K. p. 198
Brudevold, F. p. 304
Büttner, W. p. 40
Di Blasi, F. p. 317
Duckworth, R. p. 260
Eggers-Lura, H. p. 149
Emslie, R. D. p. 260
Fiorentini, S. p. 317
Grimbert, L. p. 139
Hamilton, P. M. p. 50
Hartles, R. L. p. 130
Hattyasy, D. p. 249
Henkel, G. p. 181
Hoffman-Axthelm, W. p. 12
Jackson, D. p. 80
Jenkins, G. N. p. 305
Jirásková, M. p. 209
Könlg, K. G. p. 214
Kröncke, A. p. 134
Leach, S. A. p. 119
Leonhardt, H. p. 17
Lingelbach, H. p. 193
Marthaler, T. M. pp. 109, 266
Messer, A. p. 304
Miller, J. p. 70
Mühlemann, H. R. pp. 109, 266
Nagy, Z. p. 125
Naujoks, R. p. 221
Neubauer, T. p. 221
Newesely, H. p. 174
Pigeat, G. p. 139
Porcsalmy, I. p. 125
Quentin, K. E. p. 1
Ranke, B. pp. 228, 234
Ranke, E. p. 234
Russell, A. L. p. 50
Samsahl, K. p. 275
Schait, A. p. 266
Schlatter, P. p. 109
Schützmannsky, G. p. 27
Söremark, R. p. 275
Szabó, I. p. 249
Taatz, H. p. 161
Tomić, D. p. 203
Tóth, K. p. 249
Wallis, C. P. p. 7
Welander, E. p. 58
Wespi, H. J. p. 33

ACKNOWLEDGEMENTS

ORCA wish to express their thanks to the various firms who gave financial support to aid the organisation of the London Conference and the publication of the Year Book.

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 1–6, 1961. Pergamon Press Ltd.

FLUORBESTIMMUNG IN FLUORIDIERTEM SPEISESALZ

K. E. QUENTIN

Balneologisches Institut der Universität München,
Leopoldstrasse 175, München 23, Deutschland

Zusammenfassung —Eine Fluoridierung von Speisesalz wird in Europa mit Natriumfluorid (NaF) und Dinatriummonofluorophosphat (Na_2PO_3F) durchgeführt. Einige Zeit war auch ein Speisesalz mit Calciumfluorid (CaF_2) im Handel. Analog der Trinkwasserfluoridierung ist eine analytisch-chemische Überwachung der Speisesalze erforderlich, da 100 mg Fluorid in 1 kg Salz enthalten sein sollen, entsprechend 1 mg Fluorid in 10 g Speisesalz (Tagesdosis). Genaue Untersuchungen sollten die Frage klären, wie eine chemische Kontrolle der verschieden fluoridierten Speisesalze möglich ist. Dabei ergab sich, dass in Natriumfluorid-Speisesalzen in einer 1 prozentigen Lösung ohne Destillation colorimetrisch der Fluorgehalt ermittelt werden kann. Dagegen erfordert die Fluorbestimmung des Na_2PO_3F-Salzes besondere Massnahmen (z.B. Destillation), um aus dem PO_3F^{--}-Ion das Fluor abzuspalten. Ausserdem liegt die Fluorchemikalie in unterschiedlicher Reinheit vor (90–97%). Besonders schwierig ist die Ermittlung des Fluorgehaltes im Calciumfluorid-Salz. Wegen der schlechten Löslichkeit muss nämlich das Salz selbst zur Fluorbestimmung eingewogen und dann einer Säuredestillation unterworfen werden. Einzelheiten über die aufgefundenen Fluormengen und die analytischen Kontrollverfahren werden berichtet.

Abstract — In Europe table salt is fluoridated by means of NaF, sodium fluoride and Na_2PO_3F, di-sodium mono-fluorinephosphate. For some time there also was a calcium fluoride, CaF_2 table salt on the market. Table salts should be supervised by chemical-analytical methods as is done with regard to the fluoridation of drinking water because 1 kg salt is supposed to contain 100 mg fluorine, the equivalent to 1 mg fluorine in 10 g of table salt, i.e. daily ration. Careful tests were carried out to solve the question as to how chemical control of various kinds of fluoridated table salts would be possible. It has now been found that the fluorine content can be colorimetrically detected in sodium fluoride table salts in 1% solution without distillation. On the other hand fluorine determination in Na_2PO_3F salt demands special treatment such as distillation in order to separate fluorine from the PO_3F ion. Furthermore the chemical containing fluorine appears in various degrees of purity (90–97%). It is particularly difficult to determine the fluorine content in calcium fluoride. This type of salt is far from easily soluble which entails that the salt itself has to be weighed for the determination of fluorine after which acid distillation has to be carried out. Details about the amounts of fluorine found and about the analytical control procedure will be reported.

ZUR VERSORGUNG der Bevölkerung mit ausreichenden Fluormengen wird für kariesprophylaktische Massnahmen neben Trinkwasser, Milch und Tabletten auch das Tafelsalz als Fluormedium empfohlen und benutzt. Die Angaben über den täglichen

Kochsalz-Verbrauch in den Speisen und am Tisch sind allerdings nicht einheitlich. Während WESPI (1954, 1956) mit ca. 8–10 g im Haushalt pro Tag und Kopf rechnet, beläuft sich die Menge nach Erhebungen von SANTESSON (1959) auf ca. 5–6 g. Eine für alle Länder Europas gültige Mengenangabe wird sich kaum festlegen lassen, da der Verbrauch von bestimmten, den Salzkonsum beeinflussenden Faktoren und von den Lebensgewohnheiten der Landesbewohner abhängt. Nach den Erhebungen von ZIEGLER (1957) sowie von VELICANGIL und DEMIRHINDI (1958) sind beim Salzkonsum auch die Altersstufen der Bevölkerung in Rechnung zu stellen. Immerhin kann als Richtzahl und obere Grenze für die im Handel befindlichen Speisesalze mit Fluorzusatz bei Erwachsenen ein Verbrauch im Haushalt von ca. 8–10 g angesetzt werden (WESPI). In dieser Menge soll sich demnach auch die optimale Tagesdosis an Fluor von ca. 1 mg befinden, d.h. pro Kilogramm Tafelsalz ca. 80–100 mg Fluorid. In der Schweiz enthält das fluoridierte Salz bekannterweise auch noch ca. 8 mg Jodid pro Kilogramm. Der Fluorgehalt des reinen Speisesalzes beträgt nach FELLENBERG (1949) nur ca. 0,03–0,06 mg-% und kann bei der Fluoridierung weitgehend unberücksichtigt bleiben.

Vol. 6
1961

Infolge der nicht sehr grossen Spanne zwischen optimalen und schädlichen Fluormengen ist die laufende Überprüfung der Fluorgehalte in allen Lebensmitteln mit Fluorzusatz eine unbedingte Notwendigkeit. Durch die Ausarbeitung und Empfehlung von Methoden zur Fluorbestimmung im Trinkwasser hat die ORCA diesen Gegebenheiten bereits Rechnung getragen. Wie kann nun die Fluoruntersuchung im fluoridierten Speisesalz vorgenommen werden und bestehen Unterschiede zur Wasseruntersuchung? In den verschiedenen Wassertypen hat es der Analytiker mit einer beträchtlichen Anzahl von Einzelkomponenten zu tun, die insgesamt den Mineralstoffgehalt eines Wassers ausmachen. Stets werden es mindestens 8–10 verschiedene Bestandteile ohne das Fluorid sein. Aus langjährigen Untersuchungen ist bekannt, dass verschiedene dieser Ionen eine colorimetrische oder titrimetrische Fluorbestimmung stören. In solchen Fällen muss das Fluorid als HF bzw. H_2SiF_6 durch eine Destillation isoliert werden. Erst dann kann mit dem Destillat, also einer verhältnismässig reinen Fluoridlösung, die eigentliche Bestimmung erfolgen. Bei einem fluoridierten Speisesalz sind neben den Kochsalz-Ionen Natrium (Na^+) und Chlorid (Cl^-) nur noch die Komponenten der zugesetzten Fluorchemikalie enthalten. Falls diese Komponenten die gebräuchlichen Fluorbestimmungs-Methoden nicht stören, kann in solchen Salzen die Kontrolle des Fluorgehaltes auf einfache Weise und ohne eine aufwendige Destillation vor sich gehen. Es erhebt sich also die Frage, mit welchen Fluorzusätzen in Tafelsalzen zu rechnen ist. Die nachfolgende Tabelle 1 gibt hierüber Auskunft. 3 Fluoride sind bisher bekannt geworden, die zur Fluoridierung des Tafelsalzes Verwendung fanden bzw. noch finden. Diese Verbindungen sind unterschiedlich in ihrem Fluorgehalt und in ihrer Wasserlöslichkeit. Während von Natrium—und Calciumfluorid ungefähr die gleichen Mengen zugesetzt werden müssen, um einen bestimmten Fluorgehalt des Salzes zu erzielen, wird vom Na-

triumfluorophosphat mit seinem weitaus geringeren Fluoranteil etwa die 3,5 fache Menge gegenüber Natrium- oder Calciumfluorid benötigt. Am schwerlöslichsten in Wasser ist das Calciumfluorid. Dann folgt das Natriumfluorid, und am leichtesten löslich ist das Natriumfluorophosphat. Die im Fluorschrifttum verschiedentlich

TABELLE 1. MÖGLICHKEITEN DER SALZ-FLUORIDIERUNG
Fluormenge: 100 mg Fluorid pro Kilogramm Kochsalz

Fluorzusätze zum Kochsalz	Formel	Mol.-Gew.	Fluorgehalt (%)	Löslichkeit in 100 ml Wasser
Natriumfluorid	NaF	42,00	45,24	4220 mg $^{18°C}$
Calciumfluorid	CaF_2	78,08	48,67	1,6 mg $^{18°C}$ 1,7 mg $^{26°C}$
Natriumfluorophosphat	Na_2PO_3F	143,97	13,20	25 000 mg $^{20°C}$

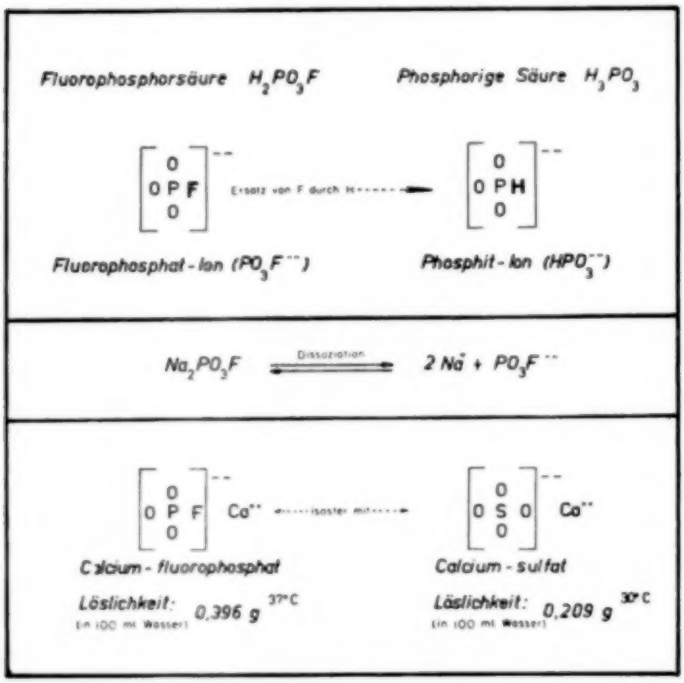
zitierte Wasserunlöslichkeit von Calciumfluorid ist nicht richtig. Die Löslichkeit von 1,6 mg CaF_2 in 100 ml Wasser entspricht immerhin 0,78 mg Fluorid! Allerdings sind es beim Natriumfluorid schon 1,91 g und beim Natriumfluorophosphat 3,3 g Fluor.

Die gute Wasserlöslichkeit des mit NaF fluoridierten Salzes und vor allem die Tatsache, dass nur die 3 Ionen Na^+, Cl^- und F^- in der Salzlösung enthalten sind, gab den Anlass zu dem Versuch, in dieser Lösung das Fluor direkt zu ermitteln. Untersuchungen mit und ohne Wasserdampf-Säure-Destillation an Modellmischungen sowie NaF-Speisesalzen des Handels führten zu den gleichen Ergebnissen und zeigten, dass bei Einhaltung einer bestimmten Konzentration der Salzlösung die vereinfachte Fluorbestimmung möglich ist. Unter der Annahme eines Fluorzusatzes von 100 mg pro kg Salz wurden 1 prozentige Salzlösungen hergestellt, die dann in 100 ml etwa 100 µg Fluorid enthielten. 20 ml dieser Lösungen mit etwa 20 µg F^- wurden im Zirkon-Eriochromcyanin-Verfahren (6) colorimetrisch getestet. Die Einhaltung der 1 prozentigen Lösung bzw. der 20 ml für den Farbtest ist deshalb wichtig, weil in dieser Menge nur 120 mg Chlorid vorliegen, die das colorimetrische Verfahren noch nicht stören. Der Fluorgehalt der untersuchten Salze lag recht genau bei 1 mg Fluor pro 10 g Salz.

Die Untersuchung eines Calciumfluorid-Speisesalzes muss dagegen auf andere Weise vorgenommen werden. Die Hauptschwierigkeit liegt in der geringen Wasserlöslichkeit dieses Salzes. Auch mit verdünnten Säuren oder Laugen, gelang es nur einen Teil des Salzes in Lösung zu bringen. Untersuchungen der gelösten und der ungelösten Salzanteile auf ihren Fluorgehalt bestätigten die Erwartung, dass sich dieser auf die Lösung und den Rückstand verteilt. Nach verschiedenen Versuchen musste dazu übergegangen werden, für jede einzelne Fluorbestimmung eine bestimmte Salzmenge einzuwiegen, in den Destillationskolben zu überführen und nach der Säuredestillation das Fluor colorimetrisch zu bestimmen. Es kann auch nur mit

einer kleinen Einwaage von 50–100 mg gearbeitet werden, da anderenfalls der hohe Chloridgehalt der Proben die Fluordestillation erheblich stört. Hier handelt es sich also um eine recht umständliche Arbeitsweise zur Kontrolle des Fluorgehaltes. Der Sinn eines mit Calciumfluorid versetzten Salzes ist überhaupt nicht recht einleuchtend, da bei der küchenmässigen Zubereitung der Speisen und der sonstigen Verwendung dieses Salzes doch mit erheblichen Fluorverlusten durch die Schwerlöslichkeit des Calciumfluorids gerechnet werden muss.

TABELLE 2



Beim Natriumfluorophosphat als drittem Fluorzusatz zu Tafelsalzen handelt es sich um eine interessante Verbindung, deren Chemismus in der nachfolgenden Tabelle 2 zusammengestellt ist. Muttersubstanz ist die phosphorige Säure H_3PO_3 mit dem Phosphit-Ion HPO_3^{--}. Das eine nicht abspaltbare Wasserstoffatom des Phosphits kann durch Fluor ersetzt werden, und man gelangt auf diese Weise zum Fluorophosphat-Ion PO_3F^{--}. Entsprechend dieser Herkunft zerfällt Natriumfluorophosphat in wässriger Lösung in Na^+- und PO_3F^{--}-Ionen. Die Empfehlung dieses Fluorzusatzes geht auf pharmakologische Untersuchungen und die gute Löslichkeit seiner Calciumverbindung zurück. Es muss ja damit gerechnet werden, dass in den Speisen

u.dgl. das PO_3F^{--}-Ion ebenso wie das F^--Ion des Natriumfluoridsalzes mit Calcium zusammentrifft. Die Löslichkeit der Calciumverbindung in Wasser beruht auf der Isosterie von Calciumfluorophosphat mit Calciumsulfat, das gleichfalls diese Wasserlöslichkeit aufweist. Mit dem Tafelsalz, das diesen Fluorzusatz enthält, lassen sich klare Lösungen herstellen. Trotzdem ist aber eine direkte colorimetrische Fluorbestimmung wie beim NaF-Salz nicht möglich. Das PO_3F^{--}-Ion reagiert mit den Farbreagenzien ganz anders als das F^--Ion. Die Färbungen mit Anteilen einer 1-prozentigen Salzlösung ergaben zunächst viel zu niedrige Werte. Beim Stehen nimmt dann die Ausbleichung der Farblösung bzw. der gemessene Fluorgehalt laufend zu und erreicht bald Werte, die weit über den theoretisch möglichen liegen. Es wäre denkbar, dass in den Untersuchungslösungen allmählich eine Hydrolyse von PO_3F^{--} in PO_4^{---} und F^- vor sich geht, und nunmehr das Phosphat den Farblack weiter ausbleicht. Diese Veränderungen verhindern auch die Bestimmung des Fluorgehaltes durch Aufstellen einer besonderen Eichkurve für Fluorophosphat. Die Ermittlung des Fluors auf indirekte Weise über den Phosphorgehalt bot keine Aussichten, da die Chemikalie in unterschiedlicher Reinheit von 90–97% Na_2PO_3F in den Handel gebracht wird und darüber hinaus Beimengungen von NaF sowie $Na_2P_2O_7$ enthalten kann. Es muss also der absolute Fluorgehalt der Chemikalie bzw. des fluoridierten Salzes ermittelt werden. Zunächst war die Frage zu klären, ob bei der Wasserdampf-Säure-Destillation eine oxydative Spaltung in F^- und PO_4^{---} erfolgt, das Fluorid quantitativ übergeht und dann in üblicher Weise colorimetriert werden kann. Zur Prüfung der Verhältnisse wurde in der Wurzschmitt-Bombe ein alkalischer Peroxyd-Aufschluss der Substanzen vorgenommen, bei dem die "Crackung" des PO_3F gewährleistet ist. Sowohl mit diesen Aufschlüssen als auch mit 0,02-prozentigen Lösungen der Chemikalie wurden Destillationen und colorimetrische Fluorbestimmungen vorgenommen. Es ergaben sich die gleichen Fluorwerte. Diese Feststellungen besagen, dass durch eine Wasserdampf-Säure-Destillation das Fluor aus dem Natriumfluorophosphat völlig abgespalten wird und quantitativ in das Destillat übergeht. Anschliessend wurden die gleichen Versuche mit dem fluoridierten Speisesalz durchgeführt. Auch hier bewährte sich wie beim NaF-Salz die Verwendung einer 1-prozentigen Lösung und die Destillation von 20 ml. Der Fluorgehalt der Natriumfluorophosphat-Chemikalie wurde mit 13% gegenüber einem theoretischen Reinheitswert von 13,20% ermittelt. Im betreffenden Kochsalz betrug der Fluorgehalt 0,85 mg in 10 g Salz*.

Zusammenfassend ist festzustellen, dass bei der Überprüfung des Fluorgehaltes in fluoridierten Tafelsalzen je nach der zugesetzten Fluorverbindung verschiedene Wege begangen werden müssen. In NaF-Salzen kann der Fluorgehalt auf einfache Weise in kurzer Zeit colorimetrisch bestimmt werden. Bei CaF_2-Speisesalzen ist

* Für das Natriumfluorophosphat und das fluoridierte Tafelsalz danke ich der Ozark-Mahoning-Company in Tulsa/USA sowie Herrn Dr. G. SANTESSON in Stockholm/Schweden.

eine abgewogene Salzmenge für die Destillation zu verwenden. Zur Fluorbestimmung im Na_2PO_3F-Salz wird ein Anteil einer Salzlösung destilliert. Alle Fluorbestimmungen erfolgen elektrocolorimetrisch. Zur Vermeidung von Störungen muss bei der Untersuchung eine gewisse Konzentration der Salzlösungen eingehalten werden.

LITERATUR

FELLENBERG, T. 1949. *Mitt. Lebensmitt. Untersuch. Hyg.* **40**, 146.
QUENTIN, K. E. und INDINGER, J. 1959. *Z. Lebensmitt. Untersuch.* **110**, 249.
SANTESSEN, G. 1959. Ber. ü.d. 6. ORCA-Kongress in Pavia.
VELICANGIL, S. und DEMIRHINDI, O. 1958. *Rev. Hyg. Med. Soc.* **6**, 251.
WESPI, H. J. 1954. *Schweiz. Med. Wschr.* **84**, 885.
WESPI, H. J. 1956. *Fluor-Vollsalz zur Kropf- und Cariesbekämpfung*, Schwabe, Basel.
ZIEGLER, E. 1957. *Landbote* Nr. 217.

Vol.
196

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 7–11, 1961. Pergamon Press Ltd.

MEASUREMENT OF CARIES PROGRESS

C. P. WALLIS
University of Edinburgh, Scotland

Vol. 6
1961

Abstract—The development of the carious process has been observed to occur with considerable regularity in large groups, but the irregular appearance of cavities in the individual has led to a general impression that caries occurs by chance. An attempt has been made to apply the group regularities to the individual subject: it is assumed only that caries is a continuous process. Using this assumption a measure of the aetiological agency can be derived which is found to be proportional under certain limited conditions to the DMF. It appears that this explains the fact that the DMF, although based on the past caries experience is also related to current caries incidence. The aetiological factors are divided into three groups related to the saliva (*a*), the tooth (*r*) and the oral morphology (*s*). The variation of these factors with age is considered.

Zusammenfassung—Es wurde beobachtet, daß das Entstehen kariöser Prozesse mit beträchtlicher Regelmäßigkeit in großen Gruppen auftritt. Aber das unregelmäßige Erscheinen von Kavitäten bei Patienten hat zu dem allgemeinen Eindruck geführt, daß Karies zufällig auftritt. Es wurde der Versuch gemacht, die Gruppenregelmäßigkeiten auf den Einzelnen anzuwenden, dabei wurde nur angenommen, daß Karies ein kontinuierlicher Prozeß ist. Unter dieser Annahme ist es möglich, ein Maß für die ätiologische Ursache abzuleiten, das sich unter bestimmten begrenzten Bedingungen mit dem DMF-Wert proportional zeigte. Dies erklärt anscheinend die Tatsache, daß der DMF-Wert auch zum heutigen Kariesauftreten in Beziehung zu bringen ist, obwohl er in früheren Karieserfahrungen begründet ist. Die ätiologischen Faktoren wurden in 3 Gruppen eingeteilt: Speichel (*a*), Zahn (*r*) und Morphologie des Mundes (*s*). Die Veränderungen dieser Faktoren mit dem Alter wurden berücksichtigt.

INTRODUCTION

REPORTS of the incidence of caries are now almost universally presented in terms of the DMF index; such a procedure has been encouraged by several interested organizations, including ORCA. The advantages of the DMF index are simplicity, a distribution which utilizes fairly evenly the entire scale, and good differentiation of experimental alterations.

On the other hand there has resulted a lack of interest in the detailed aspects of caries apparently deriving from the almost exclusive use of the DMF. In any caries survey there results a vast mass of figures which should be capable of yielding valuable information; to condense all this to a series of DMF figures is surely wasteful. Some intermediate stage of calculation which will permit both extraction and understanding of the information is clearly desirable.

The problem may be illustrated in a more specific form by considering some figures provided by a survey carried out several years ago (WALSH and SMART, 1948), which could not have been expressed in terms of the DMF (Fig. 1). This survey was

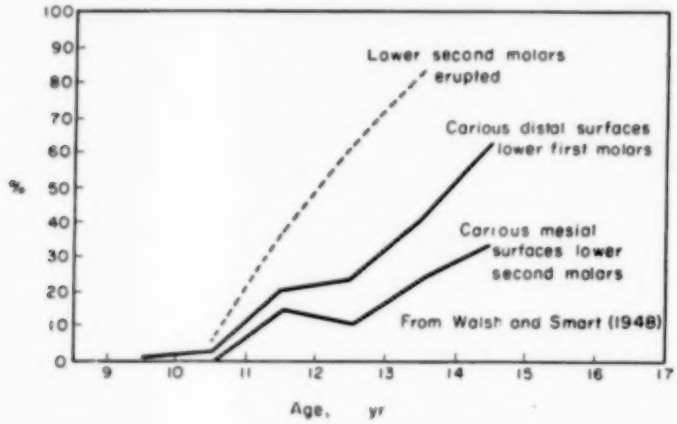


FIG. 1. Percentage of caries on opposing interstitial surfaces of lower first and second molars between the ages of 9 and 14 years. Dotted line indicates percentage of teeth erupted.

chosen because the children show a high level of conservative treatment; qualitatively similar results have been presented by BACKER DIRKS in an accompanying paper. Fig. 1 shows that the distal surface of the first molar develops little caries until the second molar erupts, when it decays rapidly. At the same time the mesial surface of the second molar also decays, but not quite so fast. From these facts one may draw certain conclusions.

The interdental space between the first and second molars is, to a first approximation symmetrical; it follows therefore that the environment and shape of the apposed surfaces are similar. Any difference in the amount of caries in these surfaces can then only be attributed to a difference in the structure of the enamel of the two teeth. This leads to the conclusion that the enamel of the second molar is more resistant to caries than the enamel of the first molar, in spite of the longer exposure of the latter to the oral fluids. One might speculate that this difference is due to the formation of the first molar at a time of greater stress.

This argument is purely qualitative; nevertheless, precise figures are available and it is reasonable to expect that one should be able to devise a quantitative argument leading to a quantitative result. In order to achieve this aim it is first necessary to describe the progress of caries in mathematical terms. A number of equations of this kind are available in the literature, but in each case the interpretation of the parameters in functional terms presents difficulties. The DMF index, on the other

hand, although purely descriptive and unsatisfactory as a mathematical tool seems to bear a remarkably close relationship to a true caries progress function, and the present work was predicated on the supposition that there is in fact a variable which can be used to describe caries progress.

THEORETICAL

There is now little doubt that the appearance of a detectable cavity is merely a stage in the development of a carious lesion. In general the development appears to proceed steadily, although there may be variations in the speed. It may therefore be concluded that the process of formation of a cavity is continuous in the mathematical sense.

Vol. 6
1961

It is postulated that the rate of increase in the size of a cavity (G) is equal to the variable (ξ) which it is desired to investigate:

$$\frac{dG}{dt} = \xi. \tag{1}$$

Integration between suitable limits gives:

$$\xi' + \xi'_0 = \frac{G}{t - t_e} \tag{2}$$

where t is the age at examination and t_e the age at eruption of the tooth and the dash indicates a mean value over the time interval in question to distinguish the function from the mean value over the population represented by a bar over the symbol. ξ' is the value of the variable attributed to the individual and is equal to zero for the individual having the same amount of caries as the population mean. The instantaneous value of ξ at the time of examination may be obtained from the equation

$$\xi = \xi' + (t - t_e)\frac{d\xi'}{dt}. \tag{3}$$

ξ'_0 is the parameter describing the population, which may change with time or with the age of the population, but all values for the same population are comparable. It represents the resultant intensity of the various aetiological factors combined to produce caries. For convenience these factors may be divided into three groups, associated with the activity of the environment (a), the morphological shape of the environment (s) and the resistance of the tooth (r). Thus

$$\xi'_0 = f(\bar{a}', \bar{r}', \bar{s}')$$

and

$$\xi' + \xi'_0 = f(a', r', s').$$

The exact form of these equations requires rather extensive data for its determination, but it may be expected that a first order relation will be satisfactory initially.

Although experiments can be devised to use G as a variable, in the normal caries survey it is a parameter of the method of examination and may be defined as the number of years required to produce a cavity of just detectable size in a mouth with unit value of the function $(\xi' + \xi'_0)$. It is unlikely that G would show a linear relation with the loss of calcium from the enamel.

RESULTS

To calculate values of ξ' it may be observed that for each value of ξ' there is a corresponding value of t. It follows therefore that the distribution of ξ' will be the same as the distribution of caries in time, and the latter shows an approximately normal distribution. The scale of ξ' can be defined as that which gives a normal distribution of ξ' and thus values of ξ' can be obtained from a table of probits.

Equation (2) predicts that ξ' is inversely proportional to t, so that if ξ' is plotted on a graph against the reciprocal of the exposure time, a straight line should be obtained, provided that the parameters remain constant.

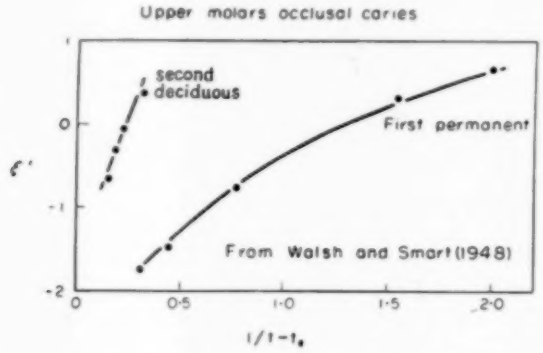


FIG. 2. Relation of ξ' to reciprocal exposure time for occulusal surfaces of first permanent and second deciduous molars between ages of 5 and 8 years.

An example is shown in Fig. 2 for the occlusal surfaces of two teeth at the same ages, but different exposure times. At longer exposure times an approximately linear relation is obtained. This does not prove that ξ'_0 is constant since the value of G probably does not correspond with the slope of the line; the change in ξ'_0 is constant however.

At shorter exposure times a curvature appears which has been found consistently in all figures examined. This curve implies that ξ'_0 shows an initial fall followed by a rise at the same time as the figure for teeth with longer exposure in the same children shows a steady rise. This must be interpreted as a steady increase in the value of $\bar{a}'$ over the period considered (5–8 years), together with a sharp increase in $\bar{r}'$ shortly after eruption tending to a more constant value after a short time.

Vol. 6
1961

The relation between ξ' and DMF has been investigated in a number of cases, of which Fig. 3 is an example. There is in general a rough linear relation in each age group, but breaks appear, particularly at DMF 4 and 8, and are more marked if particular types of teeth are considered.

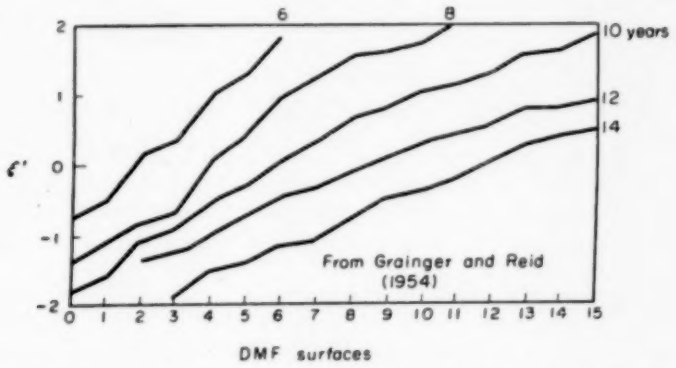


FIG. 3. Relation of ξ' to DMF surfaces between ages of 6 and 14 years.

DISCUSSION

The most pressing need in preventive dentistry at present is a method of evaluating prevention in the individual: to be able to answer the question how much caries would a child have had if he had not been treated with a particular preparation. Such a question cannot be answered by examining the mouth, but will probably require a series of special tests. Nevertheless such tests cannot be relied on in the individual unless some method of assessing individual susceptibility is available, and the relative importance of the various aetiological factors in each individual.

There is however a shortage of suitable figures on which deductions of this kind may be made. Evidence has been presented that there is an increase of enamel resistance after eruption. To give a specific value to this change it is necessary to have two further series of figures. The time of eruption of the carious teeth must be known; this will be earlier than the time of eruption of the whole tooth population, but may be calculated approximately. Secondly it is necessary to have an estimate of the rate of increase in cavity size in order to determine G, although once determined for a particular examiner the value should remain constant.

REFERENCE

WALSH, J. P. and SMART, A. 1948. *N. Z. dent. J.* **44**, 22.

Arch. Oral. Biol., Special Supplement, Vol. 6, pp. 12–16, 1961. Pergamon Press Ltd.

ACHT JAHRE KARIESSTATISTIK IN EINEM ENDEMISCHEN FLUORGEBIET

W. HOFFMANN-AXTHELM

Westdeutsche Kieferklinik, Düsseldorf, Moorenstr. 5, Deutschland

Zusammenfassung—Es wird über Beobachtungen berichtet, die in einem Zeitraum von 8 Jahren im Fluorgebiet von Berggiesshübel (Sachsen) an der dortigen Schuljugend gemacht wurden. Es wird festgestellt, dass bei gleichbleibendem Fluorgehalt des Trinkwassers eine allmähliche Zunahme der Karies und Abnahme der dentalfluorotischen Erscheinungen beobachtet wird. Es werden hierfür die verbesserten Lebensverhältnisse gegenüber der Kriegs- und Nachkriegszeit verantwortlich gemacht.

Weiterhin wird ausdrücklich festgestellt, dass Karies nicht gleich Karies zu setzen ist, da der allgemeine Verlauf der kariösen Prozesse in diesem Fluorgebiet ein erheblich gutartigerer ist als in den benachbarten Regionen.

Abstract—The report describes observations made over a period of years in the school children of a fluoride area, namely Berggiesshübel (Saxony), Germany. It has been found that at a constant fluorine content of the drinking water caries has gradually increased and dental fluorosis has decreased. Better living conditions since the war and the first years following it, are held to be responsible for this situation.

Furthermore it is emphatically stated that one kind of caries is not always the same as another because in this fluorine area the general course of the carious process is considerably slower than in neighbouring areas.

Vol. 6
1961

WIE ich auf der Genfer Tagung unserer Gesellschaft im Jahre 1955 ausführlich berichtet habe, konnten bei Untersuchungen in dem sächsischen Fluorgebiet von Berggießhübel bei einem zwischen 1 mg und 2,2 mg/l des Trinkwassers schwankenden Fluorgehalt an einem hohen Prozentsatz der untersuchten Kinder sowohl die Erscheinungen der Dentalfluorose, als auch eine wesentliche Reduktion des Kariesbefalls gegenüber Vergleichskindern in Berlin ebenso wie in fluorfreien bzw. fluorarmen Orten der Umgebung, festgestellt werden. Ich hatte Ihnen s.Z., folgende Tabelle, die sich auf insgesamt 293 Kinder bezieht, vor Augen geführt (Tabelle 1).

Der hohe Prozentsatz von zugezogenen Kindern erklärte sich damals durch die nach 1945 erfolgten Umsiedlungen, bei denen als Folge des Kriegsgeschehens eine große Anzahl von Eltern mit ihren Kindern aus den benachbarten böhmischen Gebieten eingewandert waren. Wir konnten, wie aus der Tabelle zu ersehen ist, feststellen, daß die eingeborenen Kinder im Vergleich zu den zugezogenen Kindern in erheblich höherem Maße von Dentalfluorose befallen waren, daß aber auf der anderen Seite die Kariesfreiheit der eingeborenen Kinder nicht unwesentlich über der der zugezogenen Kinder lag.

Bei eingeborenen und zugezogenen Kindern zusammengenommen ergab sich im Jahre 1952 ein Befall mit Dentalfluorose bei 57,1 Prozent und eine Kariesfreiheit bei 50,1 Prozent der Kinder. Letzteres ist ein außerordentlich glückliches Verhältnis,

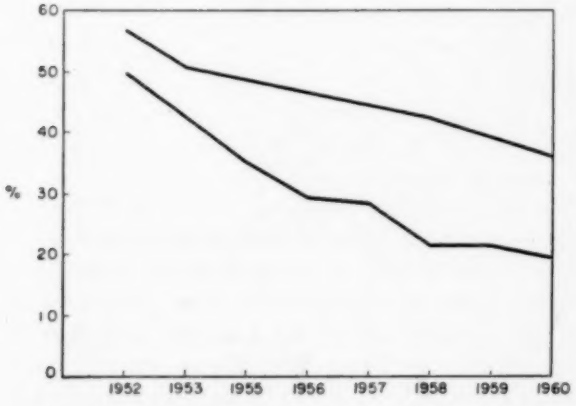
TABELLE 1. BEFALL MIT DENTALFLUOROSE UND KARIESFREIHEIT BEI IM FLUORGEBIET GEBORENEN UND ZUGEZOGENEN KINDERN

	Befall mit Dentalfluorose	Kariesfrei
Eingeborene Kinder %	80,57 (141 von 175 Kindern)	53,85 (84 von 156 Kindern)
Zugezogene Kinder %	20,34 (24 von 118 Kindern)	44,95 (49 von 109 Kindern)

TABELLE 2 UND KURVE. RÜCKGANG DES BEFALLS MIT DENTALFLUOROSE UND DER KARIESFREIHEIT VON 1952–1960 BEI ANNÄHERND GLEICHBLEIBENDEM FLUORGEHALT DES TRINKWASSERS

	Jahr der Untersuchung							
	1952	1953	1955	1956	1957	1958	1959	1960
Befall mit Dentalfluorose	57,1% v.293	50,4% v.276				41,8% v.170		35,9% v.173
Kariesfrei	50,1% v.265		35,0% v.234	29,4% v.170	28,3% v.201	21,9% v.164	22,2% v.176	19% v.174

KURVE



Oben: Dentalfluorose | Unten: Kariesfreiheit

allerdings in diesem Falle erkauft durch eine zum Teil nicht geringe kosmetische Schädigung. Wir haben nun diese Entwicklung, allerdings nicht ganz lückenlos, vom Jahre 1952 bis zum Jahre 1960 verfolgt und sind dabei zu recht interessanten Ergebnissen gekommen, die Sie aus der vorstehenden Tabelle ersehen (Tabelle 2 und Kurve).

Während wir 1952 einen Befall von 57,1 Prozent mit Dentalfluorose wahrnehmen konnten, fällt diese Kurve kontinuierlich bis zum Jahre 1960 auf 35,9 Prozent, die Kurve der Kariesfreiheit hingegen fällt von 50,1 auf 19 Prozent. Dies alles bei einem jahreszeitlich schwankenden, aber innerhalb dieser Schwankungen annähernd gleichbleibenden Fluorgehalt des Trinkwassers. Ich darf darauf aufmerksam machen, daß beide Zahlenreihen stets vom gleichen Untersucher gewonnen wurden, der Befall mit Dentalfluorose von mir, die kariesstatistischen Untersuchungen von der Schulzahnärztin Dr. Liermann. Aus diesem ebenso aufschlußreichen wie erschütternden Befund ersehen wir ein unverhältnismäßig starkes kontinuierliches Zurückgehen der Kariesfreiheit; sie ist allem Anschein nach auf die sich bessernde Ernährungslage zurückzuführen, die nach den vereinfachten Lebensbedingungen nach dem Kriege wieder den Genuß von Feinbrot, Kuchen und Süßigkeiten in den Vordergrund treten läßt.

Als Vergleich zeige ich ihnen eine Tabelle, die im Bezirk Berlin-Tiergarten vom Leiter der dortigen Schulzahnklinik Pazurek gewonnen wurde und deren Ergebnis durchaus mit den gleichen Argumenten begründet wird (Tabelle 3).

TABELLE 3. RÜCKGANG DER KARIESFREIHEIT VON 1950–1956 IN EINEM BERLINER BEZIRK

Jahr der Untersuchung Geburtsjahrgang	1950 1943	1951 1944	1952 1945	1953 1946	1954 1947	1955 1948	1956 1949
Gesamtzahl d. untersuchten Kinder	1000 unters. 1950	1000 unters. 1951	725 unters. 1952	673 unters. 1953	714 unters. 1953	740 unters. 1955	601 unters. 1956
Kariesfrei	495 = =49,5%	428 = =42,8%	176 = =24,3%	115 = =17,1%	78 = =10,9%	51 = =6,9%	38 = =6,3%

Aus diesen Zahlen, die im Jahre 1950 einen annähernd gleichen Ausgangspunkt zeigen wie die von Berggießhübel, ersehen wir einen ungleich stärkeren Abfall von 49,5 Prozent Kariesfreiheit 1950 auf 6,3 Prozent im Jahre 1956. Allerdings ist in den letzten beiden Jahren wieder ein leichtes Ansteigen um 2 Prozent zu verzeichnen gewesen, das sich vielleicht auf die bei den jüngeren Jahrgängen wieder ordnungsgemäß durchgeführte Rachitisprophylaxe mit Vitamin D-Präparaten zurückführen läßt.

Über ein Ansteigen des Kariesbefalls durch die sich bessernden Lebensbedingungen in der Nachkriegszeit berichtet auch Roos aus der Baseler Schulzahnklinik, nur sind hier die Unterschiede nicht so signifikant, wie ja auch die Differenz zwischen Mangel und Überfluß in der Schweiz nicht so extrem war wie in Deutschland.

Es stellt sich damit die Frage, wieweit unter den durch die Summe der lokalen Verhältnisse bedingten ätiologischen Faktoren, der im Gegensatz zu Berggießhübel in Berlin unterhalb einer prophylaktischen Wirksamkeit liegende Fluorgehalt des Trinkwassers für eine noch ungünstigere Entwicklung verantwortlich zu machen ist.

Im ganzen zeigt uns die Kurve, daß ein Fluorgehalt des Trinkwassers, der in der Lage ist, die Erscheinungen der Dentalfluorose hervorzurufen, die durch eine sich verfeinernde Ernährung hervorgerufene Steigerung des Kariesbefalls im Vergleich zu fluorarmen Gegenden wohl zu reduzieren, nicht aber aufzuhalten vermag.

Dieses Ergebnis langjähriger Untersuchungen müßte für jeden Befürworter einer Fluorprophylaxe deprimierend sein, ja überhaupt deren Wert in Frage stellen, wenn wir den klinischen Befund außerachtlassen würden. Karies ist eben nicht, wie in der Statistik, einfach gleich Karies zu setzen. Ganz allgemein konnten wir feststellen, daß in den fluorreichen Gegenden die Karies einen ganz anderen, mehr oberflächlichen Verlauf nimmt als in den fluorarmen Nachbargebieten. In den meisten Fällen ist der kariöse Defekt nur am Zahnschmelz zu beobachten, oder er bildet bei Dentinbeteiligung eine nicht unterminierende Kavität. Das Dentin ist dann pigmentiert und transparent, zeigt also mehr das Erscheinungsbild einer Caries sicca. Die Karies sehen wir weitaus am häufigsten als Fissurenkaries, dagegen tritt die sonst bei Jugendlichen so häufige Approximalkaries völlig zurück. Ich will aber ausdrücklich feststellen, daß gelegentlich auch solche Zähne durchaus von Karies befallen werden, welche die Erscheinungen der Dentalfluorose aufweisen, und zwar nicht etwa im Bereich des "mottled enamel", sondern an den üblichen Prädilektionsstellen. In den fluorarmen ländlichen Nachbargemeinden dagegen zeigt sich das übliche traurige Bild: der kariöse Prozeß greift schnell in die tieferen Dentinschichten über, die Kauflächen werden unterminiert, es entwickelt sich eine Caries profunda mit allen ihren Komplikationen. Wir können geradezu aus dem Zahnbefund nachweisen, ob sich im Trinkwasser ein durch das benachbarte Fluorwerk bedingter erhöhter Fluorspiegel findet oder nicht.

Diese unterschiedlichen klinischen Befunde in fluorreichen und fluorarmen Orten der gleichen Landschaft sind zwar zahlenmäßig nicht objektivierbar, sie sind aber weit eindrucksvoller als die Statistiken. Das klinische Bild, das ja im wesentlichen Richtschnur für unser Handeln sein muß, spricht einwandfrei für eine Kariesprophylaxe mit Fluoriden.

Nun zum zweiten Punkt, dem Zurückgehen der Dentalfluorose bei annähernd gleichbleibendem Fluorspiegel. Es fällt hierbei noch zusätzlich ins Gewicht, daß der Prozentsatz der dauernd Ortsansässigen laufend angestiegen ist, denn ein derartiger Zustrom von Kindern wie 1945 ist selbstverständlich nicht wieder eingetreten.

Wir haben uns auch hier um eine Erklärung bemüht: Die Tatsache, daß unter sich verbessernden Ernährungsverhältnissen und bei einem trotz jahreszeitlicher Schwankungen annähernd gleichbleibenden Fluorspiegel des Trinkwassers ein Nachlassen des Befalles von Dentalfluorose zu beobachten ist, würde die von Massler und Schour aufgestellte Theorie über die Beziehungen zwischen Dentalfluorose und Ernährung stützen. Diese Autoren hatten festgestellt, daß bei annähernd gleichem Fluorgehalt des Trinkwassers die Erscheinungen der Dentalfluorose in Italien bei unterernährten Kindern auftraten, während sie bei guternährten Kindern in den Vereinigten Staaten in wesentlich geringerer Zahl und erheblich schwächer beobachtet wurden. Sie hatten daraus geschlossen, daß die Unterernährung einen wesentlichen sekundären Faktor für das Auftreten von "mottled enamel" bildet. Es liegt damit der allerdings nicht bewiesene Schluß nahe, daß auch in dem sächsischen Fluorgebiet von Berggießhübel das Zurückgehen des Auftretens von Dentalfluorose auf die sich allmählich normalisierende Ernährungslage zurückzuführen ist.

Vol. 6
1961

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 17–26, 1961. Pergamon Press Ltd.

ERGEBNISSE DER FLUORTABLETTENZUFUHR BEI SALZBURGER SCHULKINDERN IM ALTER VON 6–10 JAHREN, 4 JAHRE NACH BEGINN DER AKTION

H. LEONHARDT

Münzgasse 2, Salzburg

Zusammenfassung—Auf Grund ausgedehnter Untersuchungen über den Einfluß von Zyma-Fluor-Tabletten bei Kindern zwischen dem 6. und 10. Lebensjahr, lassen sich nach 4 Jahren folgende Schlüsse ziehen:

1. Die Zufuhr von Fluor in Tablettenform ist auch dann sinnvoll, wenn sie erst beim Schulanfänger im 6. Lebensjahr einsetzt. Es kann dann bis zum 10. Lebensjahr eine Kariesreduktion von rund 25 Prozent (in unserem Falle 26,20 Prozent) erzielt werden.
2. Bei einem späteren Beginn—mit 7 Jahren—beträgt die Reduktion im 10. Lebensjahr rund 20 Prozent (in unserem Falle 22,40 Prozent)
3. Ein Aussetzen der Tablettengabe zwischen dem 8. und 9. Lebensjahr scheint die Resultate nicht zu beeinflußen.
4. Eine weitere Steigerung der Kariesreduktion auch nach dem 9. Lebensjahr konnte auch bei fortgesetzter Fluorzufuhr nicht beobachtet werden.
5. Ob die Wirkung der Zyma-Fluor-Tabletten auf unbestimmte Zeit auch nach Absetzen der Fluorzufuhr anhält, oder ob eine ständig fortgeführte Zufuhr zur Aufrechterhaltung der Reduktion notwendig ist, bedarf noch weiterer Untersuchungen.
6. Die Knaben sprechen in einem stärkeren Maße als die Mädchen auf die Fluorzufuhr an.
7. Eine gewisse Verzögerung des Zahnwechsels scheint durch die Fluorzufuhr gegeben zu sein.
8. Die Verzögerung ist umso stärker, je früher mit der Fluorzufuhr begonnen wird.
9. Die bleibenden Zähne scheinen bei Fluorzufuhr einen kräftigeren Schmelz zu erreichen, was sich in einem etwas verstärkten Breitenwachstum ausdrückt.

Abstract—Extensive examinations over a period of 4 years concerning the influence of Zyma Fluorine Tablets on children aged 6–10 years, justify the following conclusions:

1. The administration of tablets of fluorine is still effective if it does not start before a child of 6 years begins his life at school. Until the child reaches the age of ten, 25 per cent (in our case 26·20 per cent) caries reduction can be achieved.
2. In case of a later start—at the age of 7 years—the reduction will be approximately 20 per cent (in our case 22·40 per cent) at the age of ten.
3. It seems that omission of administration of the tablets between the ages of 8 and 9 years does not affect results.
4. In spite of continued administration of fluorine the incidence of caries did not decrease after the age of 9 years.

5. Further examinations need to be carried out in order to find whether the effect of Zyma Fluorine Tablets will last also after they are no longer taken, or whether it is necessary to continue administration in order to maintain the reduction.
6. Boys react to the fluorine intake more than girls.
7. The administration of fluorine seems to cause a certain amount of delay in the shedding of the teeth.
8. The earlier the fluorine intake starts, the more marked will be the delay.
9. It appears that the permanent teeth will attain enamel as an effect of fluorine administration which is visible in a somewhat broader appearance of the teeth.

DIE seit dem Schuljahr 1957 durchgeführte Fluortablettenzufuhr bei den Salzburger Schulkindern im Alter von 6 bis 10 Jahren, hatte folgenden Verlauf:

Da sich zur Aktion die Bevölkerung zu über 99 Prozent meldete, mußte von der üblichen Methode zweier parallellaufender Versuchsgruppen abgegangen werden und ein neuer Weg war einzuschlagen. Es wurden daher zunächst vor Beginn der Aktion alle Volksschulkinder im Alter von 6 bis 10 Jahren auf ihren Zahngesundheitszustand genau untersucht und die Ergebnisse festgehalten. Diese Resultate dienten dann als feststehende Zahl, an die die Versuchsgruppen herangeführt wurden.

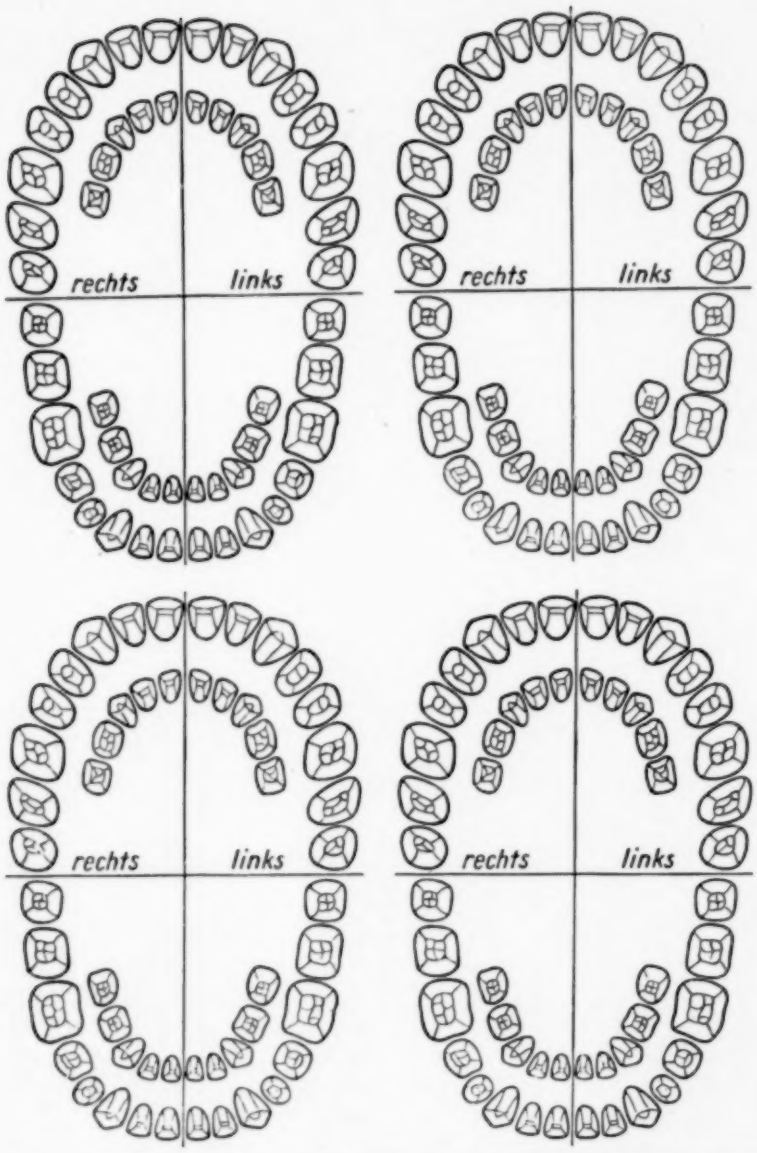
Es wurde mit der Tablettenzuteilung in der ersten und zweiten Klasse, also bei Kindern mit 6 und 7 Jahren begonnen. In jedem Falle wurden dann die Kinder, welche Tabletten erhielten, nach Klassen und Geschlecht getrennt, jährlich mit Spiegel und Sonde vom gleichen Untersucher und unter gleichen Bedingungen auf ihren Zahngesundheitszustand untersucht. Das Ergebnis wurde auf Karteikarten, wie sie die Abb. 1 zeigt, festgehalten. Dieses Karteiblatt, das von der Firma Zyma entworfen und herausgegeben wurde, hat sich uns bestens bewährt. Sie gestattet in leicht zu handhabender und übersichtlicher Form, so dass auch eine nicht besonders geschulte Assistentin damit umgehen kann, die genaue Eintragung der Anzahl, Lage und Form aller erkrankten, gefüllten und gezogenen Zähne und erlaubt aus den gefundenen Zahlen, die am Fuße des Schemas eingetragen werden, die spätere Errechnung aller notwendigen Indices.

Die Tabletten—es handelte sich um Zyma-Fluor Tabletten—wurden täglich in den Schulen von den Lehrpersonen in einer Gesamtmenge von 1 mg Fluor pro Tag und Kind ausgegeben. Für die Ferien erhielten die Schüler eine Ferialpackung mit einer entsprechenden Anzahl von Tabletten.

Jährlich wurden die Summen der gefundenen Zahlen—wie sie die Tabelle 1 zeigt—für die vorhandenen, cariösen, gefüllten und entfernten Zähne, nach Geschlecht, Milch- und bleibendem Gebiß für alle Klassen mit und ohne Tablettenzufuhr in Tabellen festgehalten. Darüber hinaus wurde die Anzahl der Kinder mit überhaupt fehlerfreiem, naturgesundem und jene mit saniertem Gebiß festgehalten und getrennt davon das Gleiche auch für das bleibende Gebiß gesondert eingetragen.

Der Unterschied ist deutlich zu erkennen. Während bei 799 Kindern ohne Tablettenzufuhr nur 54 Kinder im 10. Lebensjahr noch ein naturgesundes, blei-

Vol. 6
1961



	Anzahl	D kariös	F saniert	M extrahiert
Milchzähne				
Bleibende Zähne				

Abb. 1.

TABELLE 1. ALLE KINDER

Fluortabl. ab:	Anzahl der Kinder	Milchz.	Kariös	Gef.	Extr.	Bleib. Zähne	Kariös	Gef.	Extr.	Fehlerfr.	Saniert	Bleib. Zähne	
												San.	Fehlerfr.
I. Klasse ohne Tabl.	688	11582	3486	1051	931	3830	358	93	—	11	45	24	261
II. Klasse ohne Tabl.	775	10576	3080	1427	1599	7980	702	523	19	9	86	285	240
II. Klasse mit Tabl. ab der I. Klasse	532	7043	1707	1058	1099	5628	285	444	12	10	80	132	207
III. Klasse ohne Tabletten	741	7189	1983	1053	1682	10659	954	1160	93	7	79	329	91
III. Klasse mit Tabl. ab der I. Klasse	408	3943	941	725	807	5886	203	617	23	4	95	182	124
III. Klasse mit Tabl. ab der II. Klasse	665	6896	1687	1174	1470	9106	425	996	41	6	130	398	159
IV. Klasse ohne Tabl.	799	5414	1429	737	1576	14493	1128	1716	145	3	133	270	54
IV. Klasse mit Tabl. ab der I. Klasse	398	2928	640	566	795	6706	246	746	30	3	119	206	82
IV. Klasse mit Tabl. ab II. Klasse	429	2848	570	432	878	7554	230	934	48	5	136	246	57

Vol. 6
1961

bendes Gebiß besaßen, hatten bei 398 Kindern, die die Tabletten ab der ersten Klasse erhielten, noch 82 ein naturgesundes bleibendes Gebiß, was einer Reduktion von 20,60 Prozent entspricht. Von 429 Kindern, die erst in der II. Klasse begannen Fluortabletten zu nehmen, besaßen mit 10 Jahren noch 57 ein naturgesundes bleibendes Gebiß, was einer Reduktion von 13,28 Prozent entspricht.

TABELLE 2. ALLE KINDER

	I. Kl. ohne Tabl.	II. Kl. ohne Tabl.	II. Kl. mit Tabl. ab I. Kl.	III Kl. ohne Tabl.	III. Kl. mit Tabl. ab I. Kl.	III. Kl. mit Tabl. ab II. Kl.	IV. Kl. ohne Tabl.	IV. Kl. mit Tabl. ab I. Kl.	IV. Kl. mit Tabl. ab II. Kl.
Anzahl der Kinder	688	775	532	741	408	665	799	398	429
Kariesbefall (%)	98,40	98,84	98,1	99,06	99,02	99,10	99,63	99,25	98,84
Saniert	45	86	80	79	95	130	133	119	136
Kariesfrequenz (%)	38,40	39,60	36,34	38,80	33,73	36,13	33,98	31,37	29,72
Milchzähne (%)	47,21	57,73	54,86	65,62	62,71	62,66	69,11	68,34	65,30
bleibende Zähne (%)	11,77	15,58	13,16	20,70	14,32	16,05	20,62	15,24	16,04
d.m.f.p	7,96	7,86	7,25	6,35	6,06	6,50	4,68	5,02	4,39
d.m.f-t	2,12	1,73	1,82	1,52	1,59	1,59	1,44	1,46	1,51
D.M.F.-P	6,56	1,60	1,39	2,97	2,06	2,20	3,75	2,56	2,83
D.M.F.-T	8,76	6,40	7,58	4,84	6,97	6,22	4,84	6,56	6,24
D.m.f.-P	8,61	9,49	8,65	9,33	8,13	8,68	8,44	7,11	7,21
D.m.f.-T	2,61	2,52	2,75	2,58	2,96	2,97	2,95	3,18	3,37
Bleibende Zähne: Saniert—Naturgesund—Auf die Anzahl der Kinder bezogen									
Saniert	24	285	132	329	182	398	270	206	246
Naturgesund	261	240	207	91	124	159	54	82	57

Die Tabelle 2 veranschaulicht die Vielfalt der Zahlen und Möglichkeiten, die bei unseren Untersuchungen festgehalten und ausgewertet wurden. Neben dem Kariesbefall und der Kariesfrequenz die nach Geschlecht, nach Milch- und bleibenden Zähnen getrennt festgehalten wurden, dürften vor allem die Indices interessieren und von diesen wiederum der DMF-T Index für die bleibenden Zähne. Dieser soll auch gesondert herausgegriffen und einer näheren Betrachtung unterzogen werden. Die Tabelle 3 zeigt nun die Entwicklung des DMF-T Index für die bleibenden Zähne von der ersten bis zur IV. Klasse (6–10 Jahre).

Die oberste Kolonne gibt die Indexzahlen für die entsprechenden Klassen im Laufe der Jahre, ohne Tablettenverabreichung, mit Tablettenzufuhr ab der I. Klasse und Tablettenzufuhr ab der II. Klasse wieder.

TABELLE 3. DMFT-Index nach 1- und 2-jähriger Fluortablettengabe bei Kindern beginnend mit der I. und II. Volksschulklasse

		I. Kl. ohne Tabl.	II.Kl. ohne Tabl.	II.Kl. mit Tabl. ab I. Kl.	III. Kl. ohne Tabl.	III. Kl. mit Tabl. ab I. Kl.	III. Kl. mit Tabl. ab II.Kl.	IV.Kl. ohne Tabl.	IV. Kl. mit Tabl. ab I. Kl.	IV. Kl. mit Tabl. ab II. Kl.
	ALLE KINDER									
	DMF-T	8,76	6,40	7,58	4,84	6,97	6,22	4,84	6,56	6,24
A	Kariesreduktion zur Vergleichsklasse (%)			15,60		30,03	22,20		26,20	22,40
	Kariesanstieg zur Vorjahresparallelklasse (%)		26,94		24,37	8,05		0,00	5,90	0,32
B	Keine Tabletten in der III. Klasse		Index						6,56	
C	Gute Zahnpflege, Keine Tabletten —	Index						4,69		
	KNABEN									
	DMF-T	1,07	7,50	8,63	4,87	8,72	7,10	4,86	7,59	7,17
A	Kariesreduktion zur Klasse ohne Tabletten (%)					44,20	31,40		36,00	32,20
	Kariesanstieg zur Vorjahresparallelklasse (%)					1,30		0,20	12,90	0,864
B	Keine Tabletten in der III. Klasse DMF-T								7,69	
C	Gute Zahnpflege, Keine Tabletten— —DMF-T-Index							4,83		
	MÄDCHEN									
	DMF-T	7,23	5,57	6,71	4,78	6,06	5,64	4,85	5,96	5,63
A	Kariesreduktion zur Klasse ohne Tabl. (%)			17,00		21,10	15,30		18,60	13,80
	Kariesanstieg zur Vorjahresparallelklasse (%)		23,00		14,18	4,81		1,46	1,65	0,17
B	Keine Tabletten in der III. Klasse DMF-T-Index								5,21	
C	Gute Zahnpflege Keine Tabletten— DMF-T-Index							4,56		

B. Bei Ausbleiben der Tablettengabe in der III. Klasse.
C. Keine Tabletten bei Kindern mit besonders guter und sorgfältiger Zahnpflege.

Vol. 6
1961

Die zweite Kolonne vermerkt die Kariesreduktion in Prozenten im Vergleich der Testklasse mit der Vergleichsklasse. Es handelt sich also um die relative und nicht absolute Kariesreduktion.

In der letzten Reihe steht als Vergleich und Kontrolle sozusagen, der Endindex der IV. Klasse bei Kindern, die keine Tabletten erhielten, weil die Lehrer der Ansicht waren, daß es sich hier um Kinder mit besonders guter Zahnpflege und aus guten sozialen Verhältnissen handle und eine zusätzliche Tablettenzufuhr nicht notwendig sei. Während bei der Vergleichsklasse der Index 4,84 betrug, war er bei den sogenannten Elitekindern 4,69, was wohl die Ansicht der Lehrer widerlegen dürfte.

Ich möchte in diesem Zusammenhang hier noch auf die Tatsachen hinweisen, daß:

1. Die Kinder, bei denen die Tablettenabgabe schon in der ersten Klasse einsetzte, stärker reduzierten (26,20 Prozent) als Zöglinge bei denen die Tablettenzufuhr erst in der II. Klasse erfolgte (22,40 Prozent).
2. Das Aussetzen der Tablettenzufuhr in der dritten Klasse zwischen dem 8. und 9. Lebensjahr (vierte Kolonne), zeigt auf den Endeffekt keinen nennenswerten Einfluß.

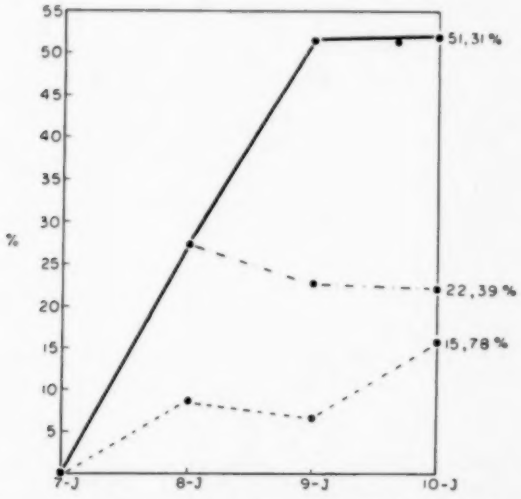


ABB. 2. Karieszuwachs der bleibenden Zähne bei Kindern zwischen dem 7. und 10. Lebensjahr. ——— Karieszuwachs ohne Tabletten; - - - - - Karieszuwachs mit Tabletten ab dem 6. Lebensjahr; - · - · - · Karieszuwachs mit Tabletten ab dem 7. Lebensjahr.

Die Knaben reagieren bedeutend stärker als die Mädchen, 36,00 zu 18,60 Prozent. Abb. 2 zeigt den Karieszuwachs noch einmal in graphischer Darstellung.

Eine weitere Tatsache, die auffällt, ist, daß die stärkste Wirkung in den ersten beiden Jahren der Fluorzufuhr erfolgt, nach dem neunten Lebensjahr aber kaum noch eine Kariesreduktion, ja eher eine langsame Zunahme wieder festzustellen ist.

Wie weit diese Zunahme anhält und ob im Laufe der Jahre vielleicht sogar die Fluorwirkung wieder ganz verloren geht, wenn sie unterbrochen und nicht fortgesetzt wird, bedarf noch weiterer Forschung.

Ehe die Schlußfolgerungen zu ziehen sind, sollen kurz noch 2 Fragen gestreift werden:

1. Wird der Zahnwechsel durch die Fluorzufuhr beeinflußt? und 2. Wird die Größe der Zähne beeinträchtigt?

Tabelle 4 zeigt die Anzahl der bleibenden Zähne pro Kind bei den 10-jährigen Schülern und Schülerinnen. Die Vergleichsgruppe ohne Tabletten, die Gruppe der Kinder mit Tabletten ab der 2. Klasse (7 Jahre) und jene Gruppe mit Tabletten ab der ersten Volksschulklasse (6 Jahre).

Vol. 6
1961

Während die Kinder ohne Tabletten im 10. Lebensjahr durchschnittlich 18,14 bleibende Zähne besaßen, hatten die Kinder mit Tabletten ab dem 7. Lebensjahr 17,60 und jene mit Fluorzufuhr ab dem 6. Lebensjahr nur 16,85 bleibende Zähne pro Kind.

TABELLE 4. EINFLUSS DER FLUORTABLETTENVERABREICHUNG AUF DEN ZAHNWECHSEL

Tablettengabe	Anzahl der bleibenden Zähne pro Kind
Alle Kinder	
Keine Tabletten	18,14
Tabletten ab der II. Klasse	17,60
Tabletten ab der I. Klasse	16,85
Knaben	
Keine Tabletten	17,36
Tabletten ab der II. Klasse	16,60
Tabletten ab der I. Klasse	15,31
Mädchen	
Keine Tabletten	18,76
Tabletten ab der II. Klasse	18,50
Tabletten ab der I. Klasse	18,17

Auch hier zeigt sich bei den Knaben eine stärkere Auswirkung in der Verzögerung des Zahnwechsels als bei den Mädchen, was auch aus der graphischen Darstellung in ABB. 3, deutlich hervorgeht.

Zur Beantwortung der zweiten Frage wurden an rund 1000 oberen, mittleren, bleibenden Schneidezähnen mit einem Meßzirkel bis auf 0,1 (Ein Zehntel) Milli-

meter genaue Messungen vorgenommen. Es wurden dabei Höhe und Breite des linken und rechten ersten oberen Schneidezahnes gemessen und der Durchschnitt errechnet.

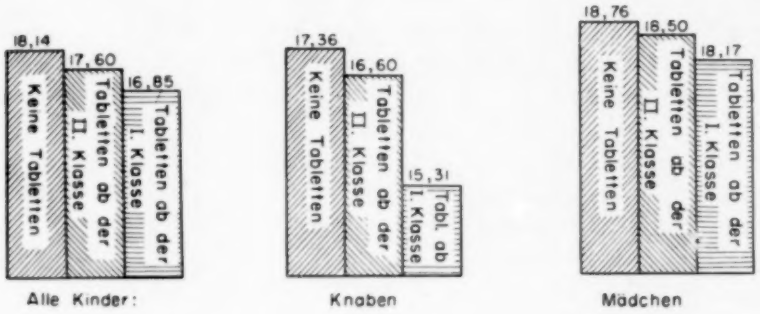


ABB. 3.

Während sich im Längenwachstum kaum ein Unterschied zwischen Test- und Vergleichsklasse feststellen ließ, zeigte sich im Breitenwachstum bei der Fluorgruppe eine geringe Verstärkung. Eigenartigerweise diesmal bei den Mädchen stärker als bei den Knaben (Tabelle 5).

TABELLE 5. EINFLUSS DER FLUORTABLETTENGABE AUF DAS LÄNGEN- UND BREITENWACHSTUM DER BLEIBENDEN, OBEREN, MITTLEREN SCHNEIDEZÄHNE (1 : 1)

	Rechts		Links	
	Länge (mm)	Breite (mm)	Länge (mm)	Breite (mm)
Alle Kinder				
Ohne Fluortabletten	9,22	8,01	8,97	7,75
Mit Fluortabletten	9,13	8,38	9,08	8,29
Knaben				
Ohne Fluortabletten	9,33	8,23	8,90	8,13
Mit Fluortabletten	9,25	8,35	9,19	8,28
Mädchen				
Ohne Fluortabletten	9,12	7,80	9,05	7,37
Mit Fluortabletten	9,02	8,42	8,97	8,30

Welche Schlußfolgerungen lassen sich nun aus diesen Ergebnissen ziehen?

Betreffend das Kariesgeschehen kann gesagt werden:

Die Fluorzufuhr in Tablettenform zeigt auch dann noch bemerkenswerte Resultate, wenn sie erst beim Schulanfänger im 6. Lebensjahr einsetzt. Es kann dann bis zum 10. Lebensjahr eine relative Kariesreduktion von rund 25 Prozent—in unserem Falle waren es 26,20 Prozent—erwartet werden.

Bei Beginn der Fluorzufuhr im 7. Lebensjahr ist die Reduktion geringer—rund 20 Prozent relative Reduktion—in unserem Falle waren es 22,40 Prozent, was die Annahme unterstreicht, daß mit späterem Einsetzen der Zufuhr auch die Wirkung absinkt.

Das zeitweilige Aussetzen der Fluorverabreichung nach dem 8. Lebensjahr, beeinflußt nach vorheriger Gabe das Resultat kaum noch. Dies läßt darauf schließen, daß nach Vollendung des 8. Lebensjahres eine bis dahin erzielte Wirkung des Fluors nicht mehr gesteigert werden kann.

Nach dem 9. Lebensjahr zeigt das Kariesgeschehen auch bei weiterer Fluorzufuhr kein weiteres Absinken mehr. Es offenbart sich vielmehr eine stationäre oder sogar eine leicht steigende Tendenz.

Ob eine Fluorwirkung auch dann noch erreicht werden kann, wenn die Zufuhr erst nach dem 10. Lebensjahr oder noch später, nach Abschluß des Zahnwechsels, einsetzt, bedarf noch weiterer Forschung.

Die Knaben sprechen in einem fast dreimal so starken Maße auf die Fluormedikation an als die Mädchen.

Die sekundäre-moralische Wirkung in Form einer verstärkten Zahnpflege ist offensichtlich. Hier werden die Mädchen stärker angesprochen als die Knaben.

Eine gewisse Verzögerung des Zahnwechsels scheint durch die Fluorzufuhr bewirkt zu werden.

Diese Verzögerung des Zahnwechsels ist umso stärker, je früher die Fluorverabreichung einsetzt.

Bei Fluorzufuhr scheinen die bleibenden Zähne ein etwas stärkeres Breitenwachstum zu haben, während das Längenwachstum fast gegenüber den fluorfreien Kindern zurückzubleiben scheint.

Die erste Tatsache würde für einen verstärkten, dichteren Schmelz sprechen, der mehr im Breiten- als im Längenwachstum zum Ausdruck kommt.

Die zweite Tatsache könnte auch mit dem etwas verzögerten Zahnwechsel in Zusammenhang gebracht werden, da bei den 10-jährigen Kindern das Zahnwachstum noch nicht abgeschlossen ist. Ein fluorbedingter, verzögerter Zahnwechsel hätte dann auch zu diesem Zeitpunkt ein Zurückbleiben des Längenwachstums zur Folge.

Vol. 6
1961

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 27–32, 1961. Pergamon Press Ltd.

SECHS JAHRE KARIESPROPHYLAXE DURCH FLUORTABLETTENGABEN

GISELA SCHÜTZMANNSKY

Harz 42/44, Halle a.d. Saale, Deutschland

Vol. 6
1961

Zusammenfassung—Sechs Jahre einer in Halle durchgeführten, jeweils bei einer grösseren Anzahl von Schulanfängern einsetzenden Fluorverabreichung rechtfertigen die Frage nach dem kariesprotektiven Erfolg dieser an die Gewissenhaftigkeit und die Konsequenz aller Beteiligten erhebliche Anforderungen stellenden Massnahme.

Als Testgruppe für die vorliegende Auswertung dienten die jetzt 12–13 jährigen, denen von 1955 ab als damaligen Schulneulingen 0,75 mg Fluor schultäglich in Tablettenform verabreicht worden waren.

Erst nach 2 Jahren war ein deutlicher Unterschied zwischen den beiden Gruppen erkennbar, sodass eine Verabreichung von Tabletten über eine kürzere Zeit wenig Wert zu haben scheint. Andererseits verlief die Erfolgskurve im 5. und 6. Jahr der Verteilung so flach, dass eine über das 10. Lebensjahr hinaus fortgeführte interne Fluoraktion in geringerem Masse erfolgversprechend scheint. Dies dürfte vor allem auf die bereits vorgeschrittene Zahnentwicklung der Probanden zurückzuführen sein. Hinzu kommt, dass in unserer Testgruppe bis zum 4. Schuljahr Unterricht und Tablettenverabfolgung in der Hand der Klassenlehrer lagen, während in den höheren Klassen bei der Unterrichtsverteilung auf viele Fachkräfte die Tablettengabe an Exaktheit der Durchführung verlor.

Auf kariöse Oberflächen aufgeschlüsselt (DMF (S)) ergab sich nach 6 Jahren eine Reduktion von 27,2 Prozent.

Abstract—In Halle, Germany, fluorine was administered to children at the beginning of their elementary school life; the test was carried out over a period of 6 years, gradually increasing the number of test cases. This justifies the question whether this test, that makes considerable demands on conscientiousness and accuracy of all the persons concerned, shows caries-protective results.

The test group under consideration is now 12–13 years old. From 1955 onwards when they were school beginners, 9·75 mg fluorine was administered to them on each school day.

A distinct difference between the two groups did not become noticeable until 2 years of the testing period had passed; therefore administration of tablets over a short period of time does not seem to have much value. On the other hand, the result over the period fifth to sixth year appears to be so small that it does not seem to promise much result if internal fluorine action would be continued after the age of 10 years. This may be due to the fact that at that time of their lives the test persons' teeth are already well developed. In addition to this we must mention that for

the first 4 school years, when one teacher looks after one form entirely by himself, the administration of tablets was under his sole control; later when several teachers were involved in the instruction of one form this situation affected the accuracy of the administration.

After 6 years we can put the reduction index of carious surfaces (DMF(S)) at 27·2 per cent.

SECHS Jahre einer ununterbrochenen peroralen Fluorverabreichung an Hallenser Schulkinder stellen mit Fug und Recht die Frage nach dem erzielten kariesprotektiven Effekt einer solchen, die Gewissenhaftigkeit und die Konsequenz aller Beteiligten in einem hohen Grade beanspruchenden Maßnahme.

Gerechtfertigt schien uns die Einleitung dieser von Anfang an auf weite Sicht angelegten Tablettenaktion im Jahre 1955 einmal durch die uns mit Sorge erfüllende ansteigende Tendenz des Kariesbefalls unter unseren Jugendlichen, zum anderen durch die geringe Fluorführung des Hallenser Trinkwassers, das mit 0,3 mg Fluor im Liter weit unter dem kariesprotektiven Optimum von 1,0 p.p.m. bleibt. Es lag also der Gedanke nahe, das bestehende Fluordefizit durch genau dosierte Tablettengaben auszugleichen, um dem pathologischen Prozeß durch prophylaktische Maßnahmen zu begegnen.

Unter Zugrundelegung der sehr genauen, Alter und Körpergewicht berücksichtigenden Berechnungsskala von HELD ergab sich für unsere in Aussicht genommene Altersgruppe der 6–7-jährigen eine tägliche Dosis von 0,75 mg Fluor, die in Form von 3 Tabletten zu je 0,25 mg gegeben wurde. Wir blieben bei dieser Dosierung mit Sicherheit innerhalb jenes Bereiches, der ein genügend hohes Fluorangebot gewährleistet, um eine maßgebliche karieshemmende Wirkung auszulösen, ohne dabei die eng umschriebene Wirkungsbreite des Fluors zu überschreiten.

Die Herstellung der Natriumfluoridtabletten übernahm nach unseren Angaben der VEB Homöopharm Dr. WILLMAR SCHWABE, Leipzig.

Mit der Ausgabe der Tabletten waren die Klassenlehrer betraut, die sich in dankenswerter Weise in den Dienst der Sache stellten. Die Verteilung erfolgte schultäglich, also unter Fortfall der Sonn- und Feiertage und der Schulferien an rund 200 Tagen im Jahre. Die Eltern waren durch ein Merkblatt über Sinn und Art der Maßnahme unterrichtet und ihr Einverständnis in jedem einzelnen Falle eingeholt worden.

Wir begannen im Januar 1955 mit der Fluortablettenversorgung bei zunächst 400 Schulanfängern der Geburtsjahrgänge 1947/48, dehnten sie im Januar 1956 auf weitere 400 Schulneulinge der Geburtsjahrgänge 1948/49 aus und in gleicher Weise auf eine annähernd entsprechende Anzahl neuer Lernanfänger zu jedem neuen Jahresbeginn. Im Verlaufe der sechsjährigen Berichtsperiode wurden einschließlich der Erstuntersuchung 7 Überprüfungen in einjährigen Intervallen durchgeführt.

Unvermeidbare Fluktuationen im Schülerbestand der Testgruppe, hervorgerufen

durch Wegzug, Klassenwechsel, Umschulung, längere Krankheiten usw., brachten eine nicht unerhebliche Einbuße an verläßlichem Untersuchungsmaterial mit sich.

Bei der vorliegenden Auswertung verfuhren wir nun so, daß wir alle Erstuntersuchungen zusammenfaßten, in gleicher Art alle Befunde nach einjähriger, nach zweijähriger, nach dreijähriger usw. Tablettenverabreichung ohne Rücksicht darauf, in welchem Kalenderjahre mit der Fluoridierung begonnen worden war. So wies zwar unsere 6 Jahre hindurch lückenlos mit Fluor versehene Gruppe nur die verhältnismäßig kleine Zahl von 258 Probanden auf, doch besaßen wir bereits 595 Befunde nach einer fünfjährigen Tablettengabe, 916 nach einer vierjährigen, 1317 nach einer dreijährigen Fluorversorgung und so kontinuierlich ansteigend in dem Maße, als jeweils ein neuer Jahrgang zum alten Stamm hinzugestoßen war.

Vol. 6
1961

TABELLE 1. REDUKTION DES KARIÖSEN NEUBEFALLS NACH FLUORTABLETTENGABEN

	Anzahl der Probanden	DMF-Zähne in % der vorhandenen bleibenden Zähne		Reduktion des kariösen Neubefalls (%)
		Fluorgruppe (%)	Kontrollgruppe (%)	
Erstuntersuchung	2493	1,7	1,7	—
Nach 1 jähriger Fluorgabe	1892	3,4	3,6	5,6*
Nach 2 jähriger Fluorgabe	1763	5,7	6,2	8,1*
Nach 3 jähriger Fluorgabe	1317	7,1	8,0	11,2
Nach 4 jähriger Fluorgabe	916	8,1	10,3	21,3
Nach 5 jähriger Fluorgabe	595	9,7	12,5	22,4
Nach 6 jähriger Fluorgabe	258	11,2	14,6	23,3

* Statistisch nicht signifikant.

Die wichtigsten Jahresergebnisse sind aus Tabelle 1 ersichtlich. Nach einem Jahre war die Reduktion an neuerkrankten bleibenden Zähnen gering. Erst nach zweijähriger Betreuung begann sich ein deutlicheres positives Ergebnis abzuzeichnen. Doch bleibt die Differenz zwischen Test- und Vergleichsgruppe mit 8,1 Prozent Verminderung des kariösen Neubefalls immer noch niedrig und ist statistisch nicht signifikant.

Nach dreijähriger lückenloser Tablettenversorgung errechnete sich eine Abnahme der Kariesrate von 11,2 Prozent. Nach vierjähriger Fluortherapie aber erfolgte ein auffallender Anstieg auf einen Reduktionswert von 21,3 Prozent gegenüber dem Karieszuwachs in der Vergleichsgruppe. Mehr als ein Fünftel der bei den Kontrollen aufgetretenen kariösen Neuerkrankungen war verhütet worden. Im 5. und 6. Jahre nahm der kariesprotektive Effekt nicht mehr im selben Maße zu. Mit 23,3 Prozent Kariesreduktion am Ende des sechsjährigen Berichtsraumes war ein Ergebnis zu

buchen, das nur unwesentlich über dem nach vier Jahren erreichten Resultat liegt.

Dieser nur noch mäßige Anstieg der Karieshemmung dürfte einmal auf das zunehmende Alter der Probanden zurückzuführen sein, zum Teil wohl auch auf gewisse Gegebenheiten innerhalb unserer Versuchsgruppen. In den ersten 4 Jahren lag der gesamte Schulunterricht in der Hand des Klassenlehrers, der auch für die Tablettenverteilung verantwortlich war. In den höheren Klassen dagegen wurde der Unterricht von einer größeren Anzahl Fachlehrer übernommen, wodurch die Tablettenausgabe leider an Exaktheit einbüßte. Auch dürfen wir uns nicht verhehlen, daß sich bei Lehrern und bei Schülern im Laufe der Jahre eine gewisse Ermüdung bemerkbar machte, die der korrekten Durchführung Abbruch tat.

Vol. 6
1961

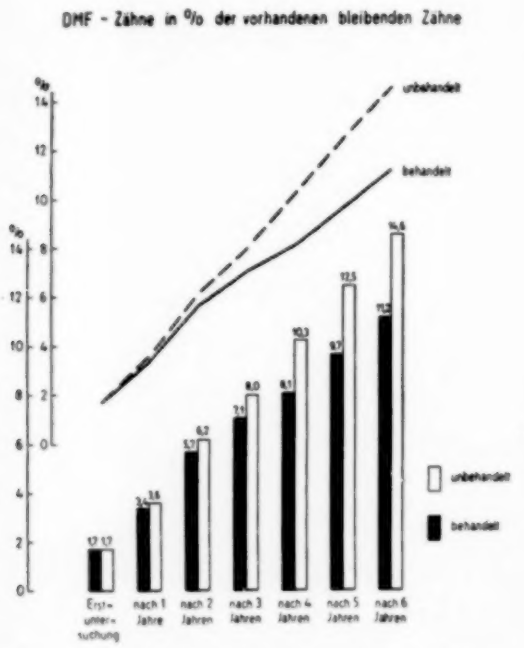


ABB. 1. DMF-Zähne in Prozent der vorhandenen bleibenden Zähne in der Fluor- und der Kontrollgruppe.

Das folgende Bild (Abb. 1) zeigt das eben Gesagte in graphischer Darstellung. Die Säulen veranschaulichen die Anzahl der DMF-Zähne bezogen auf die vorhandenen bleibenden Zähne in der Fluorgruppe und der Vergleichsgruppe, die beiden Kurven die in den 6 Berichtsjahren erzielte Verhütung des kariösen Neubefalls mit der anfänglich nur geringfügigen Karieshemmung, die erst im 3. Jahre sich deutli-

cher ausprägt, am Ende des 4. Jahres ein Maximum erreicht und sich im 5. und 6. Jahre wieder abschwächt.

Diesen mir wichtig erscheinenden Verlauf der langsam ansteigenden, dann lebhafter einsetzenden und zuletzt sich rapid mindernden Wirkung der Fluorgaben finden wir ebenso markant bei der Aufschlüsselung der Befunde auf die Zahnoberflächen (DMF(S)). Nach 2-jähriger Berichtszeit registrierten wir hier eine Kariesreduktion von 9,3 Prozent, nach vier Jahren von 24,6 Prozent, nach 6 Jahren eine solche von 27,2 Prozent. Die beiden letzten Ergebnisse sind statistisch stark gesichert und zeigen, daß die Anzahl der während der 6-jährigen Beobachtungsperiode in den Kontrollgruppen neu entstandenen kariösen Läsionen in der Testgruppe durch interne Fluorgaben um mehr als ein Viertel gesenkt werden konnte.

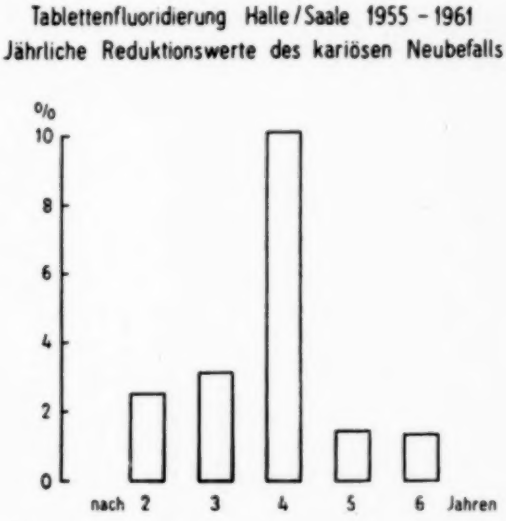


ABB. 2. Reduktionswerte des kariösen Neubefalls in den einzelnen Jahren.

Das dritte Bild (Abb. 2) schließlich legt zusammenfassend noch einmal die jährlich erzielten Reduktionswerte in unserer Testgruppe dar: den etwas zögernd einsetzenden Beginn der kariesprotektiven Wirkung, die ausgeprägte Steigerung im 4. Jahre und das Absinken auf niedrige Zuwachsraten im 5. und 6. Jahre.

So glauben wir nach den Erfahrungen an unserer Behandlungsreihe sagen zu dürfen, daß bei unseren 6-jährigen eine Tablettenbetreuung von nur 2 Jahren wenig Erfolg erwarten läßt, daß eine länger als 4 Jahre fortgesetzte Tablettengabe keine ins Gewicht fallende Erhöhung der kariesprotektiven Wirkung mehr verspricht, daß aber eine bei der Einschulung beginnende und vier Jahre lang gewissenhaft durch-

geführte perorale Fluortherapie es ermöglicht, den kariösen Neubefall im bleibenden Gebiß um etwa ein Viertel zu reduzieren. Dieser Reduktionswert änderte sich 2 Jahre nach beendeter 4-jähriger Tablettengabe nur unbedeutend.

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 33–39, 1961. Pergamon Press Ltd.

EXPERIENCES AND PROBLEMS OF FLUORIDATED COOKING SALT IN SWITZERLAND

H. J. WESPI

Department of Obstetrics and Gynecology, Aarau, Switzerland

Zusammenfassung—Im Jahre 1950 wurde fluoridiertes Speisesalz in 1-kg-Packungen mit 20 mg NaF und 10 mg KJ pro kg im Kanton Zürich/Schweiz als eine neue Art von Speisesalz eingeführt; im Jahre 1960 wurde dieses Salz in 16 von 25 Schweizerischen Kantonen verkauft. Der Gesamtverbrauch war 2 741 700 kg. 3 514 000 Schweizer, das sind 67 Prozent der Gesamtbevölkerung, haben ohne Schwierigkeiten eine Erhöhung der täglichen Fluorzufuhr erreicht, ohne Kosten und ohne irgendeinen Zwang.

Die Gesetzgebung und die Kontrolle von Nahrungsmitteln mußte diesen Zwecken angepaßt werden, so daß dieses Salz nur in jenen Gemeinden verkauft wird, in denen der Fluorgehalt des Trinkwassers niedriger ist als 0,6 mg/l. Beim Genuß dieses Salzes war der durchschnittliche Fluorgehalt der Nahrung = 1,208 mg pro Tag für eine Person im Alter von 15 Jahren. Die durchschnittliche Fluorausscheidung im Urin war 0,873 mg pro Tag für 3 Personen. Untersuchungen über den Fluorgehalt der Zähne werden zurzeit durchgeführt. Untersuchungen an neugeborenen Kindern zeigen, daß der kropfprophylaktische Effekt des Jods durch Zusatz von Fluor nicht vermindert wird. Es wäre wünschenswert, wenn einige Zahnärzte Untersuchungen über den kariesreduzierenden Effekt durchführen würden. Die Erhöhung der Zufuhr und die Kombination von fluoridiertem Salz mit anderen Arten der Fluorverabreichung wird diskutiert.

IT IS generally accepted that water-fluoridation cannot be introduced everywhere. In rural areas technical and financial difficulties make water-fluoridation impossible; in big cities political and psychological difficulties may arise. If we want to give the benefit of fluorine-prophylaxis to everybody, we have to look for other methods which might be able to replace water-fluoridation.

There are some very striking similarities between iodine-prophylaxis of endemic goitre and fluorine-prophylaxis of caries. In the first years of goitre prophylaxis, iodine was administered as tablets. In some cities, iodine was added to public water supplies. Now iodized salt, which was first started as a public health messure by my father-in-law Dr. EGGENBERGER in 1922 has shown to be the most practical form of iodine administration. That is the reason why I believe that fluoridated cooking salt might also be the most practical and effective means of fluorine prophylaxis.

I started fluoridation of cooking salt on an individual scale in 1946 (WESPI, 1948, 1950, 1954, 1956). In 1955 fluoridated salt was introduced as a "food with a prophylactic purpose" in the canton of Zürich and has now expanded over more than

half of Switzerland; the rapid development in its consumption is shown in Table 1. In 1960 sixteen of the twenty-five Swiss cantons had introduced this fluoridated and iodized salt with 200 mg of sodium fluoride and 10 mg of potassium iodide per kg. The total consumption in 1960 was 2,741,700 kg.

TABLE 1. CONSUMPTION OF FLUORIDATED SALT IN SWITZERLAND
(100 kg PER YEAR)

Canton	1955	1956	1957	1958	1959	1960
Zürich	1000	4102	4753	5546	4890	5700
Aargau		452	2112	2508	2917	3168
Schaffhausen		687	1000	1125	1125	1250
Fribourg		315	747	914	1182	1758
Solothurn			241	996	1380	1498
Uri			75	50	75	238
Luzern				1495	3007	3456
Thurgau				32	52	87
Appenzell I. Rh.				56	237	353
Appenzell A. Rh.					480	668
St. Gallen					135	4432
Obwalden					50	53
Nidwalden					15	30
Bern						4218
Zug						475
Glarus						33
Total	1000	5556	8928	12,722	15,545	27,417

Fig. 1 shows the present-day situation of fluorine-prophylaxis in Switzerland. The shaded parts are the cantons where fluoridated salt is available. In my first papers about salt-fluoridation (1947, 1950), I mentioned that it would be especially useful for the mountainous regions of Switzerland, where water-fluoridation is impossible. We have now the paradoxical situation that fluoridated salt has been introduced in the industrial parts of northern Switzerland and that the alpine cantons in the southern part still hesitate. Nr. 1 is Winterthur, where the school-milk is fluoridated and where bottles of a fluoride solution are available for individual fluoridation of the milk in every household. Nr. 2 is Aigle, the only small village in Switzerland where water-fluoridation was initiated last year. Nr. 3 is Basle, which is probably going to introduce water-fluoridation this autumn. All over Switzerland fluorine-tablets, "Zyma-Fluor" are available in pharmacies.

The fact that fluoridated salt has spread over more than half of Switzerland in less than 6 years, seems to be a proof that salt-fluoridation is considered by our authorities as a practicable means of replacing water-fluoridation because it is simple, cheap and does not include any compulsion.

Vol. 6
1961

Some weeks ago, MARTHALER (1961) completed a paper concerning the effect of fluoridated salt on the permanent teeth of children in Zürich. I am grateful to him for showing me the manuscript of his paper, which is now in press. He compared

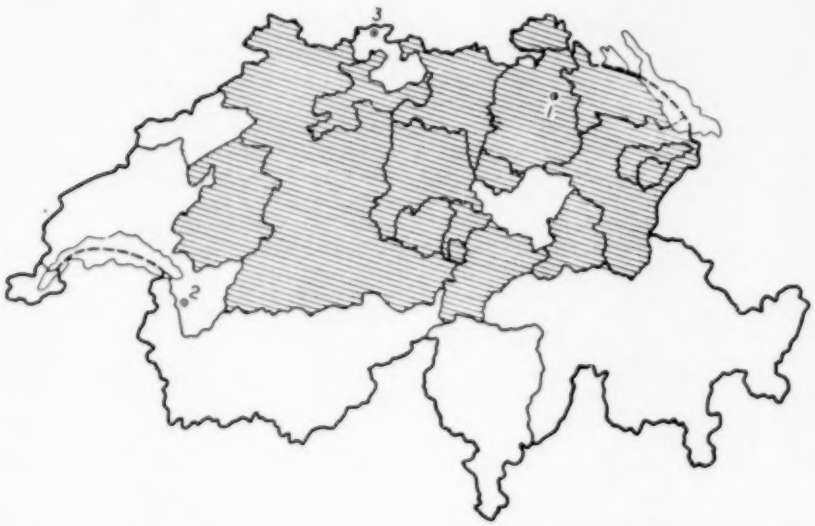


FIG. 1. Present-day situation of fluorine prophylaxis in Switzerland.
Shaded areas: Fluoridated salt available. 1: Winterthur. Fluoridation of school-milk. Fluoride solutions for individual fluoridation of milk in every household. 2: Aigle. Water-fluoridation started in 1960. 3: Basle. Water-fluoridation project.

TABLE 2. EFFECT OF WATER- AND SALT-FLUORIDATION ON PERMANENT TEETH (AFTER MARTHALER)

Grand Rapids before and after 4½ years of water-fluoridation (WF)
Zürich without or with 4½ years of consumption of fluoridated salt (SF)

	Age (years)	DMF-index		Difference	
		Before WF, without SF	After WF, with SF	DMF-teeth	Per cent
Grand Rapids	8	2·95	2·16	−0·79	−27
	9	3·90	2·48	−1·42	−36
Zürich	8–9	5·83	4·56	−1·27	−22

the DMF-Index of 8–9-year-old children in Zürich, using fluoridated salt for 4½ years with those who did not use this salt, and he compared the figures of Zürich with those of Grand Rapids (Table 2). In Grand Rapids we find a reduction in DMF-index in 8-year-old children of 27 per cent after 4½ years of water fluoridation and

a reduction of 36 per cent in 9-year-old children. In Zürich we find a reduction of 22 per cent in 8–9-year-old children using fluoridated salt. These figures are proof that fluoridated salt is not only simple and cheap, but that it is also effective. I think that the dentists should no longer insist only on water-fluoridation, but that they should also consider salt-fluoridation.

The principal problem in salt fluoridation lies in the dosage. The final solution can only be established by scientific studies with different dosages. But already at the present time we can estimate approximately the best dosage. There is a general agreement that a good fluorine-prophylaxis should give an additional fluorine supply of about 1–1·5 mg of fluorine and that it should not exceed 5–8 mg per day for an adult person.

In the village of Wädenswil, where salt-fluoridation was started, the salt consumption of every household was recorded. Table 3 gives the complete figures of

TABLE 3. CONSUMPTION OF FLUORIDATED SALT PER YEAR BY FAMILIES IN WÄDENSWIL 1957/58

Salt (kg)	No. of persons per family			
	2	3	4	5
1	—	3	1	—
2	3	10	8	1
3	3	16	36	7
4	8	23	34	18
5	8	19	41	16
6	5	16	36	23
7	3	10	24	17
8	1	7	18	13
9	4	7	13	13
10	—	2	7	10
11	—	1	7	8
12	2	2	8	5
13	—	—	4	2
14	1	—	2	6
15	—	—	3	9
16	—	1	1	2
17	—	—	—	—
18	—	—	—	—
19	1	—	—	1
20	—	—	—	—
21	—	—	—	1
22	—	—	1	—
23	—	—	—	—
24	—	—	—	2
Total	39	117	244	154

salt consumption of families with two to five persons in 1957/58. If we neglect the extreme values and consider only the 81–92 per cent of families with a reasonable salt consumption (Table 4), we find a range of 3–12 kg in families with two persons.

TABLE 4. SALT CONSUMPTION IN WÄDENSWIL 1957/58

No. persons per family	No. families examined	Percentage of families evaluated	kg/year/family		g/person/day
			Range	Mean	Mean
2	39	81	3–12	6·13	8·39
3	117	91	2– 9	5·25	4·79
4	244	92	3–12	6·22	4·26
5	154	91	4–15	8·22	4·28

Salt consumption of an adult person, g/day:	mean:	6
	normal range:	3–9
	highest value:	16

This range goes down to 2–9 kg in families of three persons. These are families with only one—mostly small—child. It goes up to 3–12 kg if another baby arrives and it goes still higher to 4–15 kg in families with five persons. The mean salt consumption in families of two adult persons is 8·39 g per day for one person. In families with children it is lower and varies between 4·26 and 4·79 g. From the experience of Wädenswil we can conclude that in Switzerland the salt consumption of a normal adult person ranges between 3 and 9 g, with a mean of 6 g, and that the highest value lies at 16 g.

TABLE 5. FLUORINE INTAKE FROM A FLUORIDATED SALT SUPPOSING A 80 PER CENT UTILIZATION (mg/day)

Daily salt consumption (g)	Fluorine dosage in the salt (mg/kg)				
	100	150	200	250	300
3	0·24	0·36	0·48	0·60	0·72
6	0·48	0·72	0·96	1·20	1·44
9	0·72	1·08	1·44	1·80	2·16
12	0·96	1·44	1·92	2·40	2·88
16	1·28	1·92	2·56	3·20	3·84

Table 5 gives the relation between fluorine content of salt, salt consumption and fluorine intake, assuming that 80 per cent of the salt remains in the food and is ingested. From this table we can see that the fluorine content should be about 200 or 250 mg per kg, if we want an additional intake of about 1 mg with a mean

Vol. 6
1961

salt consumption of 6 g. We could even go higher to 300 mg without any danger. With the maximal salt consumption of 16 g, the fluorine intake of 3·84 mg would remain lower than the dangerous limit of 5–8 mg.

With the financial support of the Swiss Academy of Medicine, Dr. FASSA* examined the fluorine content of food prepared with salt of different fluorine dosage (Table 6). If the food of a 14-year-old boy was prepared with the commercial fluoridated salt of Switzerland, its fluorine content was 1·21 mg per day. If we used a special salt with 180 mg of fluorine per kg, the fluorine content of the food wen up to 1·97 mg per day.

TABLE 6. FLUORINE CONTENT OF THE FOOD OF A 14-YEAR-OLD BOY (mg F/24 hr) (DEMOLE-FASSA)

Food prepared with fluoridated salt of different fluorine content

	Commercial fluoridated salt 90 mg F/kg	Special fluoridated salt 180 mg F/kg
Single values	1·24 1·18	1·48 2·45
Mean	1·21	1·97

In Switzerland the first stage in salt-fluoridation has been achieved. But that is only a beginning; much work has still to be done. Already now we can advise a salt-fluoridation with 200 mg of fluorine per kg and remain on this dose as long as comparative studies with different dosages will not show that a higher dosage is more effective without being harmful. Our task is now to offer a fluoridated salt with 200 mg F/kg to all individuals who want to carry out salt-fluoridation in their families and to offer fluoridated salts with different dosages for scientific studies. The problem of offering fluoridated salt to families and varying the fluorine content can be solved if every household makes its fluoridated salt individually. It is possible to produce a salt with a very high fluorine content which we can prescribe as a pharmaceutical product. We are considering producing a salt with 5200 mg F/kg; 0·1 g of this salt will contain 0·5 mg of fluorine and can be given to newborn babies and small children as a medicine. If we take 40 g of this concentrated salt and mix it with 1 kg of the usual salt, we get a fluoridated salt with 200 mg F/kg. By changing the amount of this concentrated salt, the amount of fluorine will also be changed.

I hope that in the coming weeks, this concentrated salt will be available, and that it will help to spread the use of fluoridated salt, and will help to promote scientific studies on the effect and best dosage of salt-fluoridation.

* I am obliged to Prof. DEMOLE and Dr. FASSA for their collaboration.

Vol. 6
1961

REFERENCES

MARTHALER, T. M. 1961. Zur Frage des Fluor-Vollsalzes; erste klinische Resultate. *Schweiz. Mschr. Zahnheilk.* **71**, 671-682.

WESPI, H. J. 1948. Gedanken zur Frage der optimalen Ernährung in der Schwangerschaft. Salz und Brot als Träger zusätzlicher Nahrungsstoffe. *Schweiz. med. Wschr.* **78**, 153–155.

WESPI, H. J. 1950. Fluoriertes Kochsalz zur Cariesprophylaxe. *Schweiz. med. Wschr.* **80**, 561–564.

WESPI, H. J. 1954. Fluor-Jodsalz zur Kropf- und Cariesbekämpfung. *Schweiz. med. Wschr.* **84**, 885–890.

WESPI, H. J. 1956. *Fluor-Vollsalz zur Kropf- und Cariesbekämpfung*. Benno Schwabe, Basel.

ol. 6
961

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 40–49, 1961. Pergamon Press Ltd.

EFFECTS OF SOME TRACE ELEMENTS ON FLUORIDE RETENTION AND DENTAL CARIES

W. BÜTTNER

Institut für Ernährungswissenschaft der Justus Liebig-Universität, Giessen, Germany

Abstract—The effect of the trace elements fluorine, molybdenum, vanadium, manganese and selenium on fluoride retention and dental caries experience in the white rat was studied in four experimental series. In Series I, F and Mo, separately or in combination were administered in the drinking water during pregnancy and lactation and subsequently to the offspring over a period of 120 days. The dosage levels were 50 p.p.m. F, 25 p.p.m. Mo and 50 p.p.m. Mo. The combined administration of Mo and F was significantly more effective in reducing dental caries than the administration of F alone.

When F and Mo at levels of 0·5 mg F and 1·0 mg Mo were administered by stomach tube daily after weaning over a period of 120 days (Series II), the combined administration of F and Mo was not more effective in reducing dental caries than the administration of F alone.

In Series III, F, Mo and Mn at concentrations of 50 p.p.m. each, and V at a concentration of 10 p.p.m. were administered to weaned rats in the drinking water for 90 days. The effect of these trace elements was studied for each trace element separately and in combination with F. The elements Mo, Mn and V did not significantly reduce dental caries; simultaneously administered with fluorine, these trace elements did not mediate higher caries reductions than fluorine alone.

Selenium was given as sodium selenite at levels of 5 p.p.m. and 10 p.p.m. (whole compound) in the drinking water of the mothers during pregnancy and lactation and subsequently to the offspring over a period of 120 days (Series IV). Sodium selenite increased dental caries significantly.

Fluoride retention in the femora was not changed significantly by the ingestion of Mo, Mn, V and Se.

Several trace elements other than fluorine have been studied to evaluate their influence on susceptibility to dental caries. Epidemiological studies in the human and laboratory studies in rodents indicate that Mo, V, Mn, Cu and B may reduce dental caries while Se was found to enhance dental decay. The results of several investigations in humans and rodents indicate that Mo when present in food and drinking water may be responsible for caries reduction. It was suggested that the effect of Mo on dental caries is due to a higher F-retention. Other studies suggest that similar interactions may exist between F and Mn, F and V, and F and Al. In the present study the effect of Mo, V, Mn and Se on dental caries and F-retention in the rat has been studied. It was found:

(1) The combined administration of Mo and F in the drinking water was significantly more effective in reducing dental caries than the administration of F alone;

Vol. 6
1961

(2) Se significantly increased dental caries in the rat if administered in the drinking water during the entire period of tooth formation;
(3) F-retention was not changed significantly by the administration of the trace elements studied.

INTRODUCTION

THE ACTION of various trace elements other than fluorine on dental caries has been studied in epidemiological surveys in the human and to a great extent in laboratory investigations in rodents. In 1955, HEIN reviewed the relevant literature on investigations dealing with the effect of various agents, including trace elements, on dental caries in hamsters and rats. In the meantime several other studies have been conducted with rodents to evaluate the influence of trace elements on susceptibility to dental caries. The studies by GEYER (1953) and WINIKER (1957) in hamsters and the experiments of KRUGER (1959) and TEMPESTINI and PAPPALARDO (1960) in rats suggest a caries-reducing action of vanadium. ADLER (1957) and KRUGER (1959) observed a decreased caries incidence in rats which received molybdenum. MÜNCH (1957, 1958) found in hamster assays a caries reducing effect of molybdenum and manganese. Epidemiological studies in the human by ADLER and STRAUB (1953) showed decreased caries rates in children consuming drinking water with elevated molybdenum levels. It was pointed out by LUDWIG, HEALY and LOSEE (1960) that a higher intake of molybdenum may be the prime factor responsible for a lower caries rate in 6–10-year-old children, living in an area of New Zealand with a higher molybdenum content in soil and vegetables. Epidemiological caries studies carried out by TANK and STORVICK (1960) show the influence of three trace elements on dental caries in primary and permanent teeth. Fluorine and vanadium reduced dental caries while selenium increased dental decay. This study confirms previous observations of HADJIMARKOS, STORVICK and REMMERT (1952) and HADJIMARKOS and BONHORST (1958) based on caries studies in Oregon children, which suggested that selenium may be responsible for rendering the teeth more susceptible to dental caries attack.

The effective mechanism of trace elements other than fluorine in regard to dental decay is still unknown. An interesting suggestion as to the effect of molybdenum was recently made by STOOKEY and MUHLER (1959). These authors found an increase in fluoride retention in rats receiving 50 p.p.m. of molybdenum in their drinking water from weaning over a period of 30 days. It was suggested that the increased fluoride utilization could be responsible for an anti-cariogenic action of molybdenum. In his experimental caries study KRUGER (1959) found interactions between fluorine and boron and between fluorine and aluminium. TEMPESTINI and PAPPALARDO (1960) showed in the rat that combined administration of fluorine and vanadium was more effective in reducing dental caries than the sole administration of fluorine. Another interesting finding in an epidemiological study in Mecca carried out by EL TANNIR (1959) was the probability that manganese seemed to cause a brown

coloration of mottled enamel in humans consuming a water supply with a fluorine concentration of 2 p.p.m. Manganese proved to be present in different specimens of teeth affected by mottling. The author states that "fluorine seems to exercise a"fixing" action in that respect".

In order to discover some further experimental evidence for these suggested interactions, the purpose of this study was to determine the effects of the trace elements molybdenum, manganese, vanadium and selenium on dental caries and fluoride retention in the rat.

EXPERIMENTAL METHOD

The experiment consisted of four series, all of which were conducted with caries-susceptible rats of the Wistar strain. The cariogenic coarse stock corn diet was composed of (in per cent): yellow corn grits, 52·7; ground yellow corn, 11·3; powdered whole milk, 30; alfalfa, 4·8; iodized sodium chloride, 1; irradiated yeast, 0·2. The diet, distilled drinking water and the drinking solutions containing the respective trace elements were given *ad libitum*. The rats were housed in pairs in raised screen metal cages. For the preparation of the desired aqueous solutions the following reagent grade (Merck) compounds were used: NaF; $(NH_4)_6\,Mo_7\,O_{24}\cdot 4\,H_2O$; $MnCl_2\cdot 4\,H_2O$; $NH_4\,VO_3$; and Na_2SeO_3. In comparison with distilled water, no change in pH occured in the concentrations used.

Caries evaluation at the end of each respective experimental period was carried out according to the method described by MUHLER, NEBERGALL and DAY (1954). Fluorine analysis followed the directions of the ORCA standard method described by QUENTIN and INDINGER (1959).

Series I. Six groups of five female rats each were mated and received during pregnancy and lactation drinking water solutions of: (1) distilled water as controls, (2) 25 p.p.m. Mo, (3) 50 p.p.m. Mo, (4) 50 p.p.m. F, (5) 25 p.p.m. Mo + 50 p.p.m. F, (6) 50 p.p.m. Mo + 50 p.p.m. F. The same solutions were administered to the respective offspring over a period of 120 days, after which time the progeny were sacrificed for caries evaluation and fluorine analysis in the femora.

Series II. A total of forty male rats, weighing between 110 and 120 g were equally divided into four groups receiving in 1 ml of aqueous solution daily by stomach tube: (1) H_2O (control), (2) 1·0 mg Mo, (3) 0·5 mg F, (4) 1·0 mg Mo + 0·5 mg F. After 120 days of administration, caries was evaluated and fluorine was determined in the femora and in the pooled samples of whole maxillary incisors.

Series III. 144 male rats, weighing between 40 and 50 g, were equally divided into eight groups receiving the following concentrations of trace elements in their drinking water: (1) H_2O (control), (2) 50 p.p.m. Mo, (3) 50 p.p.m. Mn, (4) 10 p.p.m. V (5) 50 p.p.m. F, (6) 50 p.p.m. Mo + 50 p.p.m. F, (7) 50 p.p.m. Mn + 50 p.p.m. F, (8) 10 p.p.m. V + 50 p.p.m. F. The experimental period lasted 90 days, after this time caries was evaluated and fluorine determined in the femora. 120 rats completed the experiment.

Series IV. In this part of the investigation the effect of selenium on dental caries was studied: selenium was administered in the drinking water during the period of tooth formation. Three groups of eight female rats each were mated and received during pregnancy and lactation drinking solutions of: (1) H_2O (control), (2) 5 p.p.m. Na_2SeO_3, (3) 10 p.p.m. Na_2SeO_3. The offspring received the same concentrations of sodium selenite for 120 days. The dosage levels used partly inhibited reproduction. Therefore, only twenty pups were born in Group 2, and seven pups in Group 3, as compared with thirty-one pups in the control group. The offsprings were sacrificed after 120 days, and again caries was estimated and fluorine analysed in the femora.

RESULTS

The findings are summarized in Tables 1–8. Table 1 shows the effect of the administration of fluorine and molybdenum in the drinking water to the mothers and subsequently to the offspring over a period of 120 days on the dental caries expe-

TABLE 1. EFFECT OF PRENATAL AND POSTNATAL ADMINISTRATION OF MOLYBDENUM AND FLUORINE ON DENTAL CARIES IN RATS

F and Mo in drinking water 120 days	No. of rats	Mean No. of carious molars per rat	Mean No. of carious lesions per rat	t
Control	15	4·5±0·2†	7·2±0·6†	
25 p.p.m. Mo*	8	4·5±0·6	7·7±1·1	
50 p.p.m. Mo	14	4·1±0·3	6·9±0·6	
50 p.p.m. F*	12	2·2±0·5	3·0±0·8	4·4
25 p.p.m. Mo 50 p.p.m. F	10	1·1±0·3	1·2±0·3	1·8
50 p.p.m. Mo 50 p.p.m. F	8	0·5±0·2	0·6±0·3	2·2

* Compounds used: $(NH_4)_6\,Mo_7\,O_{24} \cdot 4\,H_2O$.
NaF.
† Standard deviation.

rience. The concentrations of 25 p.p.m. Mo and 50 p.p.m. Mo did not result in significant caries reduction. A marked reduction of 58 per cent in the mean number of carious lesions was found in rats receiving 50 p.p.m. F in their drinking water as compared with the controls. The combined administration of fluorine and molybdenum was significantly more effective in reducing dental caries than the administration of fluorine alone. There was a dosage response according to the two levels of 25 p.p.m. and 50 p.p.m. Mo plus 50 p.p.m. F. The differences in mean number of carious lesions per rat between control group and 50 p.p.m. F-group and between 50 p.p.m. F-group and 50 p.p.m. Mo + 50 p.p.m. F-group are statistically signi-

ficant, the *t*-values being 4·4 and 2·2, respectively. As seen from the Table 2 data, simultaneous ingestion of fluorine and molybdenum did not result in an increased fluoride retention in the femora. Similarly, rats of the group receiving 50 p.p.m. Mo and no additional fluoride did not retain increased levels of fluorine as compared with the control rats.

TABLE 2. EFFECT OF PRENATAL AND POSTNATAL ADMINISTRATION OF MOLYBDENUM AND FLUORINE ON FLUORIDE RETENTION IN THE FEMORA OF RATS

F and Mo in drinking water 120 days	No. of rats	F in femur*	
		Concentration (p.p.m.)	Total -F
Control	15	69±3†	22±0·9† μg
25 p.p.m. Mo	8	42±2	15±0·7 μg
50 p.p.m. Mo	14	78±9	24±2·5 μg
50 p.p.m. F	12	5860±270	2·0±0·06 mg
25 p.p.m. Mo 50 p.p.m. F	10	5860±256	2·2±0·07 mg
50 p.p.m. Mo 50 p.p.m. F	8	5910±204	2·0±0·10 mg

* Ash.
† Standard deviation.

In Series II fluorine and molybdenum was administered daily by stomach tube. Applying this procedure, a caries reduction was found in the fluorine groups only, however, combined ingestion of fluorine and molybdenum did not result in higher caries reductions than in rats with a daily ingestion of 0·5 mg F. Again, fluoride retention in the femora was not changed by the consumption of molybdenum.

TABLE 3. EFFECT OF ADMINISTRATION OF MOLYBDENUM AND FLUORINE BY STOMACH TUBE ON DENTAL CARIES IN THE RAT

F and Mo by stomach tube daily 120 days	No. of rats	Mean No. carious molars per rat	Mean No. carious lesions per rat
H_2O (control)	10	4·1±0·3†	7·3±0·7†
1·0 mg Mo*	9	3·8±0·5	6·5±1·1
0·5 mg F*	8	2·6±0·4	3·1±0·5
1·0 mg Mo 0·5 mg F	9	3·0±0·5	4·0±0·7

* Compound used: $(NH_4)_6\ Mo_7\ O_{24} \cdot 4\ H_2O$
NaF.
† Standard deviation.

Vol. 6
1961

These data are shown in Tables 3 and 4. The maxillary whole incisors of each group were pooled, powdered and ashed and fluorine determined in duplicates. This procedure did not allow the calculation of the standard deviation. The mean values show that similar to the femora no increased fluoride retention in incisor teeth resulted from the ingestion of molybdenum.

TABLE 4. EFFECT OF ADMINISTRATION OF MOLYBDENUM AND FLUORIDE BY STOMACH TUBE ON FLUORIDE RETENTION IN THE FEMORA AND INCISORS OF THE RAT

F and Mo by stomach tube daily 120 days	No. of rats	Final weight (g)	F in femur*		F in whole incisor† (p.p.m.)
			Conc. (p.p.m.)	Total (μg)	
H_2O (control)	10	348±12‡	151±2‡	53±2‡	43
1·0 mg Mo	9	350±10	161±2	57±2	55
0·5 mg F	8	333±8	2550±43	905±13	248
1·0 mg Mo 0·5 mg F	9	343±14	2479±70	891±21	235

* Ash.

† Ash, maxillary incisors of whole group pooled.

‡ Standard deviation.

The results of Series III of the study are summarized in Tables 5 and 6. As compared with the control group, caries of the molar teeth of rats weighing between 40 and 50 g and receiving for 90 days in their drinking water 50 p.p.m. Mo, 50 p.p.m. Mn and 10 p.p.m. V, respectively, was not significantly reduced. Significant reductions in the mean number of carious lesions were obtained in all fluoride groups, however, without any additional effect if trace elements were administered simultaneously with fluorine. Weight gains in all groups were similar. Neither in rats consuming molybdenum, manganese, or vanadium, nor in rats consuming these trace elements together with fluorine, did significant changes occurred in fluoride storage of the femora.

The administration of 5 p.p.m. and 10 p.p.m. sodium selenite in the drinking water of the mothers and subsequently to the offspring over a period of 120 days resulted in a significant increase of dental caries as compared with the controls. As judged by the mean numbers of carious lesions, the incidence of caries of the 5 p.p.m. Na_2SeO_3-group was 26 per cent higher, that of the 10 p.p.m. Na_2SeO_3-group 38 per cent. The extent of carious lesions in the two selenium groups was increased 38 and 54 per cent, respectively. Unfortunately, the selenium levels used reached a toxic range reducing reproduction and weight gain significantly. Again, fluoride concentration in the femora was not changed. Total fluoride was reduced in the femora of the selenium groups. This effect is probably due to the lowered food ingestion of these animals. The data of Series IV are shown in Tables 7 and 8.

TABLE 5. EFFECT OF ADMINISTRATION OF Mo, Mn, V AND F IN THE DRINKING WATER OF WEANING RATS ON DENTAL CARIES

Administered 90 days in drinking water	No. of rats	Mean No. carious molars per rat	Mean No. carious lesions per rat
Control	14	4·6±0·3*	8·6±0·6*
50 p.p.m. Mo	13	4·5±0·3	8·0±0·4
50 p.p.m. Mn	12	4·2±0·2	7·7±0·8
10 p.p.m. V	17	4·0±0·1	7·4±0·5
50 p.p.m. F	17	3·6±0·3	5·3±0·5
50 p.p.m. Mo 50 p.p.m. F	15	3·5±0·3	5·2±0·5
50 p.p.m. Mn 50 p.p.m. F	16	3·9±0·3	6·0±0·7
10 p.p.m. V 50 p.p.m. F	16	3·7±0·3	5·9±0·6

* Standard deviation.
Compound used: $(NH_4)_6\ Mo_7\ O_{21} \cdot 4\ H_2O$
$MnCl_2 \cdot 4\ H_2O$
$NH_4\ VO_3$
NaF.

TABLE 6. EFFECT OF ADMINISTRATION OF Mo, Mn, V AND F IN THE DRINKING WATER OF WEANING RATS ON FLUORIDE RETENTION IN THE FEMORA

Administered 90 days drinking water	No. of rats	Final weight (g)	F in femur*	
			Concentration (p.p.m.)	Total (μg)
Control	14	337±12†	135±6†	48±2†
50 p.p.m. Mo	13	326±10	126±2	44±1
50 p.p.m. Mn	12	331±7	141±3	46±3
10 p.p.m. V	17	330±11	136±3	48±2
50 p.p.m. F	17	327±10	4420±146	1560±63
50 p.p.m. Mo 50 p.p.m. F	15	330±11	4320±242	1520±61
50 p.p.m. Mn 50 p.p.m. F	16	315±6	4360±125	1470±99
10 p.p.m. V 50 p.p.m. F	16	319±8	4080±110	1450±32

* Ash.
† Standard deviation.

TABLE 7. EFFECT OF PRENATAL AND POSTNATAL ADMINISTRATION OF SODIUM SELENITE ON DENTAL CARIES IN THE RAT

Se in drinking water 120 days	Sex	No. of rats	Wt. gain in 120 days	Mean No. carious lesions*	t†	Extent of carious lesions	t†
Control	m	17	330±9‡	5·3±0·5‡		11·6±1·4‡	
	f	14	207±7				
5 p.p.m. Na_2SeO_3	m	13	240±5	7·2±0·7	2·5	18·7±2·2	3·0
	f	7	176±7				
10 p.p.m. Na_2SeO_3	m	5	220±18	8·6±1·1	2·8	25·2±3·2	4·0
	f	2	148±3				

* Sexes combined.

† t-Values as compared with the control group.

‡ Standard deviation.

TABLE 8. EFFECT OF PRENATAL AND POSTNATAL ADMINISTRATION OF SODIUM SELENITE ON FLUORIDE RETENTION OF THE FEMORA IN THE RAT

Se in drinking water 120 days	No. of rats	F in femur*	
		Concentration (p.p.m.)	Total (μg)
Control	31	152±2†	52±1†
5 p.p.m. Na_2SeO_3	20	155±2	45±1
10 p.p.m. Na_2SeO_3	7	165±5	41±2

* Ash.

† Standard deviation.

DISCUSSION

Difficulties arise in summarizing the literature reports of the effects of trace elements other than fluorine on dental caries in rodents. Differences in species, strains, cariogenic diets, dosage levels and mode of administration make it nearly impossible to compare one study with another. Furthermore, in cases of effectiveness it is difficult to decide whether the cation, the anion or the compound itself was effective. In the present study none of the trace elements, except for fluorine, was found to be effective in reducing dental caries in the rat. The combined administration of molybdenum and fluorine was effective only if both elements were available during tooth formation and subsequently in the drinking water of the experimental animals. Similar effects of a synergism between molybdenum and fluorine were reported recently by ROBERTS (1961). The author administered molybdenum and fluorine at levels of 25 p.p.m. in the drinking water to weaning rats and found after 130 days

Vol. 6
1961

the highest caries reduction in those rats receiving both elements. Increased fluoride levels in the skeleton of rats receiving molybdenum as reported in the studies of STOOKEY and MUHLER (1959, 1960) and of ROBERTS (1961) were not found in our animals. In accordance with the findings of MUHLER (1957), vanadium administration in the drinking water of weaning rats was ineffective in reducing dental decay. While manganese was shown to be effective in hamsters (MÜNCH, 1958), no significant reduction of caries in the rat was observed by KRUGER (1959) and in the present study.

The relation of selenium to dental caries susceptibility has been discussed recently (EDITORIAL, 1959). Here it was pointed out that no conclusive statement can be made regarding a causative relationship between selenium and dental caries until studies are conducted of selenium administration during tooth development. The results of the present study indicate that selenium, if received during tooth development, enhances dental decay in the rat. In experiments reported by WHEATCROFT, ENGLISH and SCHLACK (1951) and by MUHLER and SHAFER (1957) no cariogenic effect of selenium was observed; however, in these studies weaning rats had been used. It may be possible that selenium causes structural changes in developing dental hard tissues, as observed by ENGLISH (1949) in the teeth of dogs. Further investigations in these laboratories are dealing with a more detailed evaluation of the mechanism of selenium with regard to dental caries.

If one considers the numerous discrepancies found in animal experiments dealing with the effect of trace elements on dental caries, one may conclude that, in all probability, except for fluorine, none of the trace elements is of vital importance for preventive dentistry. On the other hand epidemiological caries studies in children indicate that at least molybdenum, vanadium and selenium should be studied more closely in order to reach a clear understanding of their mechanism in dental decay.

Acknowledgements—The author wishes to express his thanks to Mr. B. TEUCHER for his valuable help during the study and to Mrs. S. SCHÜLKE and Miss S. SOYKA for their technical assistance.

REFERENCES

ADLER, P. and STRAUB, J. 1953. A water-borne caries-protective agent other than fluorine. *Acta Med., Acad. Sci. Hung.* **4**, 221–225.

ADLER, P. 1957. Experiments with albino rats upon the caries-protective effect of water-borne molybdenum. Proceedings of the 4th Congress of ORCA, Malmö. *Odont. Revy*, 48–53.

EDITORIAL. 1959. Selenium in teeth. *Nutr. Rev.* **17**, 205–207.

EL TANNIR, M. D. 1959. Mottling of enamel in Mecca and the Arabian Peninsula. A survey and research study carried out in Saudi Arabia. *Amer. J. Publ. Hlth* **49**, 45–52.

ENGLISH, J. A. 1949. Experimental effects of thiouracil and selenium on the teeth and jaws of dogs. *J. dent. Res.* **28**, 172–194.

GEYER, C. F. 1953. Vanadium, a caries-inhibiting trace element in the Syrian hamster. *J. dent. Res.* **32**, 590–595.

HADJIMARKOS, D. M. and BONHORST, C. W. 1958. The trace element selenium and its influence on dental caries susceptibility. *J. Pediat.* **52**, 274–278.

HADJIMARKOS, D. M., STORVICK, C. A. and REMMERT, L. F. 1952. Selenium and dental caries. *J. Pediat.* **40**, 451–455.

HEIN, J. W. 1955. Effect of various agents on experimental caries. A résumé. *Advances in Experimental Caries Research* (Ed. by SOGNNAES, R. F.), pp. 197–222. American Academy for the Advancement of Science, Washington, D. C.

KRUGER, B. J. 1959. The effect of "trace elements" on experimental dental caries in the albino rat. *Univ. Queensland Papers, Dept. Dent.* **1**, 1–28.

LUDWIG, T. G., HEALY, W. B. and LOSEE, F. L. 1960. An association between dental caries and certain soil conditions in New Zealand. *Nature, Lond.* **186**, 695–696.

MUHLER, J. C. 1957. The effect of vanadium pentoxide, fluorides, and tin compounds on the dental caries experience in rats. *J. dent. Res.* **36**, 787–794.

MUHLER, J. C., NEBERGALL, W. H., DAY, H. G. 1954. Studies on stannous fluoride and other fluorides in relation to the solubility of enamel in acid and the prevention of experimental dental caries. *J. dent. Res.* **33**, 33–49.

MUHLER, J. C. and SHAFER, W. G. 1957. The effect of selenium on the incidence of dental caries in rats. *J. dent. Res.* **36**, 895–896.

MÜNCH, J. 1957. Die Beziehungen des Spurenelementes Molybdän zur Zahnkaries. Proceedings of the 4th Congress of ORCA, Malmö. *Odont. Revy*, 48–53.

MÜNCH, J. 1958. Die Rolle des Spurenelementes Mangan bei der Kariesprophylaxe. Proceedings of the 5th Congress of ORCA, Bruxelles. *J. dent. Belge* 284–290.

QUENTIN, K.-E. and INDINGER, J. 1959. Beiträge zur Analytik kleiner Fluormengen in Lebensmitteln und Wässern—III. Die quantitative Fluoridbestimmung. *Z. Lebensmitt Untersuch.* **110**, 249–260.

ROBERTS, R. A. 1961. The synergistic effect of molybdenum and fluoride upon caries in the rat. (Abstr.). 39th General Meeting of I.A.D.R., Boston. *J. dent. Res.* In press.

STOOKEY, G. K. and MUHLER, J. C. 1959. Effect of molybdenum on fluoride retention in the rat. *Proc. Soc. Exp. Biol.*, N.Y. **101**, 379–380.

STOOKEY, G. K. and MUHLER, J. C. 1960. Effect of molybdenum on fluoride retention in the rat. *J. dent. Res.* **39**, 671, (Abstract).

TANK, G. and STORVICK, C. A. 1960. Effect of naturally occuring selenium and vanadium on dental caries. *J. dent. Res.* **39**, 473–488.

TEMPESTINI, O. and PAPPALARDO, G. 1960. The combined action of vanadium and fluorine on experimental caries in the white rat. *Panminerva Med.* **2**, 344–348.

WHEATCROFT, M. G., ENGLISH, J. A. and SCHLACK, C. A. 1951. Effects of selenium on the incidence of dental caries in white rats. *J. dent. Res.* **30**, 523–528.

WINIKER, M. 1957. Weitere Versuche zur Kariesbekämpfung mit Vanadinverbindungen. Proceedings of the 4th Congress of ORCA, Malmö. *Odont. Revy*, 42–47.

Vol. 6
1961

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 50–57, 1961. Pergamon Press Ltd.

DENTAL CARIES IN PERMANENT FIRST MOLARS AFTER EIGHT YEARS OF FLUORIDATION

A. L. RUSSELL and PEGGY M. HAMILTON

National Institute of Dental Research, National Institutes of Health, United States Public Health Service, Bethseda 14, Md., U.S.A.

INTRODUCTION

TO HAVE practical value a caries-inhibitory agent must be effective against the attack of caries on the first permanent molar. Treatment for these teeth is ordinarily the prime service in a dental program for children. The nature and extent of inhibition of molar caries following fluoridation of the community water is, hence, a matter of first importance in any assessment of the practicability of this measure, but relatively few data of this type have been reported. Four years after fluoridation of the community water in Newburgh, New York, there was a definite increase in the numbers of caries-free first molars (AST, FINN and CHASE, 1951). After 8 years caries in these teeth had been reduced by half (AST *et al.*, 1955). At the end of the 10 year term of study the evidence indicated that proximal surfaces of molars might have been somewhat better protected than occlusal surfaces (AST *et al.*, 1956). Twelve years after fluoridation at Evanston, Illinois, carious first molars had been decreased by about one-half in children aged 12–14 years, with lesions on proximal surfaces reduced by one-half to three-quarters (HILL, BLAYNEY and WOLF, 1961).

This paper is a report of an independent study in Maryland. It is a description of patterns and magnitude of caries attack in permanent first maxillary and mandibular molars, in the mouths of children aged 5–15 years, 8 years after fluoridation of their domestic water. The study population, and methods employed, have been described in detail (RUSSELL, 1956 a, b; RUSSELL and WHITE, 1959). All of the children reported upon were born in one of the two counties adjacent to the District of Columbia, and have lived in this area ever since. None of the children has received such caries-preventive treatment as the topical application of fluoride solutions. Their water was fluoridated with sodium fluosilicate to a level of 1·0 p.p.m. F in January 1952 and minimal enamel changes have been observed in a few of the younger children. Of the cases considered to be fluorosis about nine out of ten were classified as questionable, with the remainder in the very mild category. None were unsightly; virtually all changes were so slight as to be detectable only by an examiner skilled in the diagnosis of the condition.

Inspections were made by a small and carefully-standardized group of examiners using mirror and explorer, under ample and uniform artificial light. The 8 year examinations described here were carried out in January and February 1960, and findings are compared with data from a similar examination made in January and February 1952.

At the outset essentially all decayed, missing or filled permanent teeth in children 9 years of age or younger were first molars. For the entire age span of 5–15 years, first molars comprised 60·7 per cent of all DMF teeth. Since fluoridation, total numbers of DMF teeth have been reduced by about two-thirds in children

TABLE 1. DENTAL CARIES IN MARYLAND CHILDREN AT THE OUTSET, AND AFTER 8 YEARS OF FLUORIDATION

Age at last birthday (years)	Number examined		Mean number of DMF* teeth per child	
	1952	1960	1952	1960
5	60	47	0·03	0·00
6	171	243	0·41	0·09
7	211	270	1·09	0·45
8	181	246	1·90	0·57
9	223	243	2·42	1·02
10	199	226	3·09	1·69
11	191	227	4·39	2·41
12	228	470	4·96	3·14
13	233	503	6·15	4·27
14	188	385	7·66	5·28
15	65	140	8·57	5·80

* Decayed, missing or indicated for extraction because of caries, or filled permanent teeth.

who have used the fluoride water from birth, by about one-half in children 1–3 years of age at the time of fluoridation, and by about one-third in children 4 years of age or older at that time (Table 1). This necessarily entailed sweeping reductions in the numbers of carious first molars.

CARIES IN FIRST MOLAR TEETH

Differences are most marked in children 5–8 years of age in 1960. These children were born after fluoridation. In 8-year-olds examined in 1960 about nine of ten maxillary first molars, and about five of six mandibular first molars, remained caries-free (Table 2). Nearly half of these teeth had been carious in the 8-year-old group examined in 1952. Lesser differences were seen in children aged 9–11 years in 1960, who first used a fluoride water during the calcification period of the first molar teeth; slight differences in children aged 12–14 years, who first

used a fluoride water after calcification but before eruption of these teeth; and somewhat greater total in children aged 15 years, whose water had been virtually free of fluoride until most of their first molars were in eruption and at risk of caries.

TABLE 2. CARIES-FREE FIRST MOLAR TEETH IN 1952 AND IN 1960

Age group	First fluoride exposure, 1960 children	Percentages of first molar teeth in eruption which were caries-frree			
		Maxillary		Mandibular	
		1952	1960	1952	1960
5	At birth	100	100	91	100
6		89	99	81	95
7		80	91	63	86
8		58	89	47	83
9	During calcification period	50	79	40	72
10		42	64	28	56
11		38	51	18	46
12	After calcification, before eruption	37	43	20	33
13		32	32	18	25
14		23	25	16	17
15	After eruption	25	20	20	14

TABLE 3. MEAN NUMBERS OF DMF FIRST MOLARS PER CHILD IN 1952 AND IN 1960

Age group	Maxillary first molar			Mandibular first molar		
	1952	1960	Difference (%)	1952	1960	Difference (%)
5	0·00	0·00	—	0·03	0·00	−100
6	0·14	0·02	−86	0·27	0·07	− 74
7	0·36	0·18	−50	0·72	0·28	− 61
8	0·83	0·22	−73	1·05	0·33	− 69
9	0·99	0·43	−57	1·20	0·57	− 53
10	1·17	0·72	−38	1·43	0·88	− 38
11	1·23	0·98	−20	1·63	1·07	− 34
12	1·27	1·14	−10	1·59	1·33	− 16
13	1·44	1·35	− 6	1·64	1·49	− 9
14	1·55	1·50	− 3	1·69	1·65	− 2
15	1·51	1·59	+ 5	1·60	1·72	+ 8

Despite some fluctuation due to sampling error, mean numbers of DMF first molar teeth per child show essentially the same patterns (Table 3). There are marked differences between the 1952 and 1960 findings in children aged 5–8 years, lesser differences in children aged 9–11 years, and essentially none in children aged 13 years or older.

CARIES IN FIRST MOLAR SURFACES

When findings are computed for DMF first molar surfaces rather than teeth the differences between 1952 and 1960 are somewhat greater (Table 4), particularly at the intermediate ages. These differences in pattern are clearly evident when observed numbers are contrasted with the numbers of DMF first molar teeth and

TABLE 4. MEAN NUMBERS OF DMF FIRST MOLAR SURFACES PER CHILD IN 1952 AND IN 1960

Age group	Maxillary first molar			Mandibular first molar		
	1952	1960	Difference (%)	1952	1960	Difference (%)
5	0·00	0·00	—	0·03	0·00	−100
6	0·15	0·02	−87	0·30	0·08	− 73
7	0·44	0·21	−52	0·90	0·35	− 61
8	1·07	0·26	−76	1·50	0·49	− 67
9	1·48	0·60	−59	2.02	0·87	− 57
10	1·79	1·05	−41	2·85	1·34	− 53
11	2·24	1·47	−34	3·94	1·97	− 50
12	2·53	1·90	−25	4·11	2·71	− 58
13	2·93	2·62	−11	4.36	3·64	− 17
14	3·39	3·29	− 3	4·72	4·10	− 13
15	3·08	4·01	+30	4·97	5·29	+ 6

TABLE 5. EXPECTED AND OBSERVED NUMBERS OF DMF FIRST MOLARS AND DMF FIRST MOLAR SURFACES IN THE 1960 STUDY GROUP

Age group	Number expected	Number observed	Difference (%)
DMF first molar teeth			
5– 8	849	280	−67
9–11	1757	1071	−39
12–14	4161	3806	− 9
15	436	463	+ 6
DMF first molar surfaces			
5– 8	1095	360	−67
9–11	3297	1675	−49
12–14	10,060	8168	−19
15	1124	1301	+16

surfaces which would have appeared had the 1952 attack rates persisted without change (Table 5). In children aged 5–8 years caries totals have been reduced by about two-thirds according to either method of estimation. In children aged 9–11 years, however, 39 per cent fewer DMF teeth are affected in 49 per cent fewer

surfaces. In children aged 12–14 years a negligible difference in numbers of DMF teeth is associated with a difference of practical significance—19 per cent—in the numbers of DMF surfaces. Children aged 15 years in 1960 showed evidence of caries experience somewhat higher than their 1952 counterparts by either method of assessment.

Caries was active principally in pit and fissure areas in children aged 5–8 years in 1960. In these children occlusal lesions, with or without extension into the lingual pit, made up 98 per cent of carious surfaces in maxillary first molars. Occlusal lesions with or without extension into the buccal pit made up 92 per cent of carious

TABLE 6. EXPECTED AND OBSERVED MEAN NUMBERS OF DMF FIRST MOLAR SURFACES PER CHILD, 1960

Age group	Children examined	Occlusal		Lingual		Buccal		Mesial		Distal		All surfaces	
		E*	O†	E*	O†	E*	O†	E*	O†	E*	O†	E*	O†
Maxillary first molar													
5– 8	806	0·40	0·13	0·06	0·02	0·03	0·00	0·01	0·00	0·02	0·00	0·52	0·16
9–11	696	1·08	0·67	0·25	0·18	0·22	0·06	0·16	0·08	0·12	0·04	1·82	1·03
12–14	1358	1·38	1·30	0·47	0·44	0·31	0·24	0·48	0·40	0·31	0·19	2·95	2·56
15	140	1·51	1·59	0·46	0·71	0·34	0·50	0·51	0·75	0·26	0·46	3.09	4·01
Mandibular first molar													
5– 8	806	0·58	0·16	0·03	0·01	0·18	0·11	0·03	0·01	0·03	0·01	0·85	0·29
9–11	696	1·28	0·76	0·28	0·09	0·63	0·34	0·42	0·11	0·30	0·08	2·91	1·38
12–14	1358	1·58	1·42	0·51	0·34	0·89	0·75	0·85	0·57	0·61	0·38	4·44	3·45
15	140	1·52	1·68	0·67	0·74	1·03	1·06	0·95	1·05	0·77	0·76	4·94	5·29

* Mean numbers which would have been observed had the 1952 attack rates persisted without change.

† Mean numbers actually observed.

surfaces in the mandibular first molar (Table 6). These taken together averaged 1·22 DMF surfaces per child in 1952 against 0·42 per child in 1960, a difference of about 66 per cent. A substantial inhibition of caries in these surfaces—from a mean of 3·24 per child to a mean of 1·95, or about 40 per cent—was seen in children aged 9–11 years. Observed and expected means for these pit-and-fissure surfaces were essentially the same in children aged 12 years or older.

In proximal surfaces, however, together with areas in which caries usually attacks a smooth surface—the buccal surface of the maxillary and the lingual surface of the mandibular first molar—there was evidence of a useful inhibition up to the age of 14 years. In children aged 12–14 years there was an average of 2·12 DMF smooth surfaces per child against an expected mean of 3·07—a difference of 31 per cent, or a saving of 1293 surfaces in the 1358 children examined. There were fewer

DMF pit-and-fissure surfaces in this group by only about 9 per cent. First fluoride exposure in these children came after calcification of the first molars was complete, but before these teeth had erupted.

No inhibitory effect of any sort could be demonstrated in children aged 15 years, i.e. those in whom most first molars had erupted and were at risk of caries before fluoride was first added to the domestic water. The 1960 means are, in fact, uniformly higher than those seen in 1952. But comparison on this basis is limited to the sixty-five children 15 years of age who were examined in 1952 and, against this narrow base, the observed differences are well within sampling error limits.

Vol. 6
1961

DISCUSSION

Studies based upon this general design—the "before and after" type—have sometimes been questioned on the grounds that conclusions are *post hoc* and lacking in control. In this instance excellent internal control has been provided by teeth in eruption at the time of fluoridation. Analyses from time to time have shown that such teeth have continued to decay, throughout the term of study, at rates equal to or somewhat higher than those observed in 1952 (RUSSELL, 1956b, RUSSELL and WHITE, 1959). This continues to be true of first molars in children aged 15 years in 1960. It is clear that no unknown factor, such as a dentifrice or a change in diet or oral hygiene, has reduced caries in teeth actually standing in the mouth over the course of this study.

In sharp contrast, DMF means for first molar teeth and surfaces of children aged 5–8 years in 1960 are substantially lower than those seen in children of the same age in 1952. Further, these teeth are continuing to decay at rates drastically lower than those observed at the outset of the study. In 1952, for example, occlusal surfaces of mandibular first molars in children aged 5–8 years were decaying at the rate of about 22 per cent of available surfaces per year, i.e. in the course of any one year about 22 per cent of mandibular occlusal surfaces, caries-free at the outset, would become carious. The 1960 attack rate in this surface, in children of these ages, is about 5 per cent. This is a difference (Table 7) of about three-quarters;

TABLE 7. CARIES ATTACK RATES IN PERMANENT FIRST MOLARS, 1952 AND 1960, CHILDREN AGED 5–8 YEARS

	1952	1960	Difference (%)
Maxillary first molar			
All surfaces	4·02	0·92	−77
Occlusal surfaces	16·54	3·89	−76
Mandibular first molar			
All surfaces	5·56	1·84	−67
Occlusal surfaces	22·34	5·19	−77

about the same reduction in attack rates has occurred in maxillary first molar occlusal and total surface. The reduction in the total mandibular surfaces attack rate is somewhat smaller, due principally to the persistence of caries in the buccal pit, but this rate is about two-thirds less than the rate in 1952. There is no indication in these data that the inhibitory effect will be attenuated with the passage of time.

Techniques used in this estimate of caries attack rates are inapplicable to older children examined in 1960, since uniformity of experience between independent groups is assumed. Caries rates are still dropping in cohorts of children aged 9 years or older in 1960 (RUSSELL and WHITE, 1959).

SUMMARY

After 8 years use of a fluoridated water:

(a) Children aged 5–8 years, with use of a fluoridated water from birth, apparently exhibit the ultimate degree of inhibition. Numbers of DMF first molar teeth and surfaces were about two-thirds lower than in children of the same ages at the beginnings of fluoridation. Of the restorations still required by this group, about nineteen out of twenty were pit-and-fissure fillings. Their teeth continue to decay at rates about three-quarters less than those observed in children of these ages before fluoridation was begun.

(b) There was a considerable inhibition of first molar caries in children aged 9–11 years, with first use of a fluoridated community water during the calcification period. They exhibited about 40 per cent fewer DMF first molar teeth, and about 49 per cent fewer DMF first molar tooth surfaces, than children of the same ages at the outset of fluoridation.

(c) Children aged 12–14 years, with first fluoride exposure after calcification but prior to eruption of the permanent first molars, showed about the same caries experience in pit and fissure areas as children of the same ages before fluoridation, but caries in smooth surface areas was less by about 31 per cent.

(d) No inhibition could be demonstrated in the small group of children aged 15 years. Most of their first molars were in eruption and at risk of caries before fluoridation of the community water.

REFERENCES

AST, D. B., BUSHEL, A., WACHS, B. and CHASE, H. C. 1955. Newburgh-Kingston caries-fluorine study—VIII. Combined clinical and roentgenographic dental findings after eight years of fluoride experience. *J. Amer. dent. Ass.* **50**, 680–685.

AST, D. B., FINN, S. B. and CHASE, H. C. 1951. Newburgh-Kingston caries fluorine study—III. Further analysis of dental findings including the permanent and deciduous dentitions after four years of fluoridation. *J. Amer. dent. Ass.* **42**, 188–195.

AST, D. B., SMITH, D. J., WACHS, B. and CANTWELL, K. T. 1956. Newburgh-Kingston caries-fluorine study—XIV. Combined clinical and roentgenographic dental findings after ten years of fluoride experience. *J. Amer. dent. Ass.* **52**, 314–325.

HILL I. N., BLANEY, J. R. and WOLF, W. 1961. Evanston dental caries study twelve years lates. *Dent. Progr.* **1**, 95–99.

RUSSELL, A. L. 1956a. Oral health study in children of suburban. *Publ. Hlth Rep., Wash.* **71**, 626–632.

RUSSELL, A. L. 1956b. Longitudinal technics in the study of oral disease. *Amer. J. Publ. Hlth* **56**, 28–35.

RUSSELL, A. L. and WHITE, C, L. 1959. Dental caries in Maryland children after 5 years of fluoridation. *Publ. Hlth Rep., Wash* **74**, 289–295.

ol. 6
961

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 58–69, 1961. Pergamon Press Ltd.

PROBLEMS OF RECORDING AND SAMPLING IN CLINICAL INVESTIGATIONS OF CARIES

E. WELANDER

Royal Dental School, Stockholm, Sweden

Abstract—In the statistical analysis of the results of clinical caries investigations, the method of registration and the choice of observation unit cause a great many difficulties. In recent years attention has been paid to the method error which may be both random and systematical.

It is obvious that statistical methods assume a central importance in the study of odontological problems, and applied to clinical investigations of caries the statistical analysis shows typical features. The natural observation unit in caries registration is the tooth or the surface, while the sampling unit usually is the individual (the whole dentition). The statistical analysis must, therefore, take into account the interdependence between the observation units (the teeth). This is most easily effected by calculating a caries index for each subject, expressed for example in DMF teeth or in the number of intact teeth. It should, however, be pointed out that the accuracy of different caries indices is more or less unreliable, since they are affected by such factors as previous dental care.

In certain types of investigation the question of sampling may be even more complicated, e.g. in studies of the effect of caries inhibiting agents. In such investigations it is not unusual that the case material consists of children from different schools. The question then arises whether the child or the school should be considered as the observation unit. There is a possibility that children from one school present a caries status quite different from that shown by children from another school. If that is the case there would be a certain interdependence between children from the same school, a circumstance which must be taken into account when applying the analysis of statistical significance.

Zusammenfassung—Bei der statistischen Analyse der Ergebnisse von klinischen Kariesuntersuchungen verursacht die Protokollierungsmethode und die Auswahl der Beobachtungsgruppe große Schwierigkeiten. In den letzten Jahren wurde dem methodischen Fehler, der sowohl zufällig wie auch systematisch sein kann, Beachtung geschenkt.

Es ist offensichtlich, daß statistische Methoden eine zentrale Bedeutung bei odontologischen Problemen haben. Auf die klinischen Untersuchungen angewandt, zeigt die statistische Analyse typische Grundzüge. Die natürliche Beobachtungseinheit bei der Kariesregistrierung ist der Zahn oder dessen Oberfläche, während die Stichprobeneinheit im allgemainen der Patient ist (das ganze Gebiß). Die statistische Analyse muß deshalb die gegenseitige Abhängigkeit der beiden Beobachtungswerte berücksichtigen. Das geschieht am einfachsten durch die Berechnung eines Kariesindex für jede Person, z.B. in Form des DMF-Wertes oder ausgedrückt durch die Zahl der gesunden Zähne. Man sollte jedoch darauf hinweisen, daß die Genauigkeit der einzelnen Kariesindices mehr oder weniger unzuverlässig ist, da sie durch Faktoren wie die Zahnpflege beeinflußt wird.

Bei gewissen Untersuchungsarten ist die Frage der Stichprobenauswahl sogar noch komplizierter, z.B. bei Untersuchungen über den Effekt von karieshemmenden Substanzen. Bei solchen Untersuchungen ist es nicht unüblich, daß das Material aus Kindern verschiedener Schulen besteht. Es erhebt sich dann die Frage, ob das Kind oder die Schule als Beobachtungseinheit betrachtet werden soll. Es besteht die Möglichkeit, daß Kinder einer Schule einen anderen Kariesstatus zeigen wie Kinder einer anderen Schule. Wenn das zutrifft, gäbe es eine gewisse Gemeinsamkeit der Kinder einer Schule, ein Umstand, der in Betracht gezogen werden muß, will man eine Analyse der statistischen Signifikanz durchführen.

Vol. 6
1961

INTRODUCTION

DURING the last decade there has been a vigorous intensification of work in all branches of caries research.

In the field of caries statistics praiseworthy attempts have been made in some quarters to create some acceptable order in the technical terminology and to work out suitable statistical methods for scientific caries research. These attempts have not always brought us any nearer to the solution of the problems, and we have to face the fact that there still remains much to be done in this field.

As regards the technical terminology appertaining to the clinical diagnosis of caries the sub-committee on standardization of caries recording methods, F.D.I., has presented us with an international programme. It is obviously a great advantage for future work to have obtained standard terms covering the various conceptions of caries. This is not sufficient, however, and does not mean that statistical investigations of caries from now on will give the desired results with any greater accuracy than hitherto. The real problems are in fact encountered when one actually launches out on scientific caries investigations.

In our attempts to solve caries problems the statistical method naturally assumes a central position. In planning investigations it is therefore necessary to study the special demands which have to be made, firstly on the planning of the investigation, and secondly on the analysis of the results. It must be quite clear what general conclusion are to be expected and what difficulties might arise in the interpretation of the results.

It must also be clear what special demands are to be considered in the planning, depending on whether the investigation is to be descriptive or analytical, in the same way as in static or dynamic analysis. In the planning it is also important to know what methods of statistical analysis are available and to have a clear idea of how to carry out the statistical analysis of the material—this may be done by making up so-called blind Tables. Time does not permit a deeper penetration into detail, and I will confine myself to two main points:

(1) The method error in caries recording and some aspects of its consequences in the planning of the research work;

(2) The choice of units for recording and statistical analysis of significance.

THE METHOD ERROR IN CARIES RECORDING AND SOME ASPECTS OF ITS CONSEQUENCES IN THE PLANNING OF RESEARCH WORK

In caries recording the method error is apparent in instances where two dentists have recorded the caries status of the same patient at about the same time. The error is shown up by different degrees of deviation between the recordings of the same tooth. Judging from the reported results of clinical investigations the method error has not been sufficiently considered, though it ought to be well known in these days (GYTHFELDT, 1938; RADUSCH, 1941; BERGGREN and WELANDER, 1960b). There still seems to be an adherence to the misleading opinion that a levelling-out of the method error will take place if the case material is sufficiently large. This opinion would be justified if the method error in caries recording were only a chance happening. This does not, however, seem to be the case. In an investigation which will be described below, the method error was proved to be systematic. This means that some examiners always made too few recordings, others too many, in relation to the mean number, and the terms "over-recorder" and "under-recorder" therefore seem to be justified.

The question of the method error was studied in great detail in an investigation carried out at school dental clinics in Stockholm. In connexion with the clinical investigation which is being carried out at ten schools in Stockholm for the purpose of establishing the effect of topical application of various fluoride compounds, a study of the recording error was also made in order to ascertain how the ten school dental officers participating in the investigation differed in their manner of recording carious lesions. In this study the ten dentists examined the same ten patients, all 10 years of age. The recording was done at the Department of Pedodontics at the Royal School of Dentistry in Stockholm, the examiners working under identical conditions. Their findings were noted on special charts by the nurses assisting them. As was expected there were considerable differences in the manner of detecting and recording caries. It should be pointed out that of the first five patients in Table 1, for special reasons only one half of the dentition was recorded.

Table 1 shows the number of teeth with primary lesions recorded by the ten examiners. The figures in the bottom line show a range from 11 to 26. If the surface was used as the observation unit instead of the tooth, a still wider dispersion was found, as seen in Table 2.

In a clinical investigation with probe and mirror it is often difficult to detect incipient caries or very small lesions on surfaces not easily accessible owing to adjacent teeth. It is equally difficult to decide whether a fissure is carious or not. In order to make a closer study of the recording of fissure caries only this type of caries was entered in Table 3.

This Table shows that there were also large discrepancies between the figures given by the different examiners in the case of fissure caries only (lowest 1, highest 8 cavities).

TABLE 1. NUMBER OF TEETH WITH PRIMARY LESIONS RECORDED BY TEN EXAMINERS

Patient	Examiner									
	I	II	III	IV	V	VI	VII	VIII	IX	X
1. M. T.	1	2	2	2	0	3	1	2	3	1
2. J. N.	0	1	1	1	0	1	1	1	1	1
3. O. S.	0	1	1	1	0	1	1	1	1	0
4. M. J.	0	0	0	0	0	0	1	0	0	0
5. L. J. J.	2	3	5	2	2	2	4	5	2	2
6. L. B.	3	1	4	1	0	2	2	1	2	2
7. I. S.	5	3	8	10	3	6	6	5	6	3
8. E. Ö.	3	2	3	3	4	3	4	2	6	2
9. E. K.	1	1	2	4	2	3	1	1	1	1
10. S. S.	1	0	0	2	0	4	1	1	2	0
No. of teeth with primary lesions	16	14	26	26	11	25	22	19	24	12

TABLE 2. NUMBER OF SURFACES WITH PRIMARY LESIONS RECORDED BY TEN EXAMINERS

Patient	Examiner									
	I	II	III	IV	V	VI	VII	VIII	IX	X
1. M. T.	1	2	2	2	0	3	1	2	3	1
2. J. N.	0	1	1	1	0	1	1	1	1	1
3. O. S.	0	1	1	1	0	1	1	1	1	0
4. M. J.	0	0	0	0	0	0	1	0	0	0
5. L. J. J.	2	4	5	3	3	3	5	6	3	2
6. L. B.	3	1	4	2	0	2	2	1	3	3
7. I. S.	5	3	8	11	3	6	6	5	6	3
8. E. Ö.	3	2	3	3	4	3	4	2	6	2
9. E. K.	1	1	3	6	2	3	1	1	1	1
10. S. S.	1	0	0	2	0	4	1	1	2	0
No. of surfaces with primary lesions	16	15	27	31	12	26	23	20	26	13

TABLE 3. NUMBER OF CARIOUS FISSURES RECORDED BY TEN EXAMINERS

Patient	Examiner									
	I	II	III	IV	V	VI	VII	VIII	IX	X
1. M. T.	1	0	1	1	0	2	1	1	1	1
2. J. N.	0	0	0	0	0	0	0	0	0	0
3. O. S.	0	0	0	0	0	0	0	0	0	0
4. M. J.	0	0	0	0	0	0	0	0	0	0
5. L. J. J.	0	0	0	0	0	0	0	0	0	0
6. L. B.	1	0	2	1	0	2	2	1	2	1
7. I. S.	2	0	1	3	2	0	0	1	1	2
8. E. Ö.	1	1	1	1	1	2	1	1	1	1
9. E. K.	0	0	0	2	0	0	0	0	0	0
10. S. S.	0	0	0	0	0	2	1	1	2	0
No. of teeth with fissure caries	5	1	5	8	3	8	5	5	7	5

Five of the ten examiners recorded five fissure cavities each which might have indicated close agreement had it not been for the fact that the cavities were recorded in different teeth (Table 4).

TABLE 4. DISTRIBUTION OF FISSURE CARIES ACCORDING TO TOOTH

Patient	Examiner									
	I	II	III	IV	V	VI	VII	VIII	IX	X
1. M. T.	−6	0	−6	−6	0	−05 −6	−6	−6	−6	−6
2. J. N.	0	0	0	0	0	0	0	0	0	0
3. O. S.	0	0	0	0	0	0	0	0	0	0
4. M. J.	0	0	0	0	0	0	0	0	0	0
5. L. J. J.	0	0	0	0	0	0	0	0	0	0
6. L. B.	05+	0	05+ 6+	05+	0	+05 05+	+05 05+	05+	+05 05+	05+
7. I. S.	5+ −4	0	5+	5+ 4+	5+ −4	0	0	5+	−4	5+ −4
8. E. Ö.	6+	6+	6+	6+	6+	6+ −6	6+	6+	6+	6+
9. E. K.	0	0	0	4+ 4−	0	0	0	0	0	0
10. S. S.	0	0	0	0	0	6+ +5	+5	5+	+5 5+	0

In order to improve the clinical examination with probe and mirror the use of X-ray examination has been proposed. Earlier investigations (LUNDBERG and MARKEN, 1958) have shown, however that also in X-ray examinations there is an observation error. To ascertain how the ten school dental officers differed in their interpretation of the X-ray pictures, a special study was made. Full mouth roentgenograms of five of the patients were studied by the examiners with regard to carious lesions. The results are shown in Table 5 which makes clear that the dentists differed greatly in their interpretations. The range is from 2 to 19.

TABLE 5. NUMBER OF CARIOUS LESIONS RECORDED BY TEN EXAMINERS FROM FULL MOUTH ROENTGENOGRAMS OF FIVE PATIENTS

Patient	Examiner									
	I	II	III	IV	V	VI	VII	VIII	IX	X
L. B.	1	1	1	0	1	1	2	1	2	1
E. K.	1	1	1	0	1	1	1	1	1	1
I. S.	1	1	6	0	4	3	7	2	1	6
S. S.	0	0	2	0	0	0	0	0	0	0
E. O.	5	6	3	2	13	6	9	9	4	5
Total	8	9	13	2	19	11	19	13	8	13

For a closer study of the differences between the ten examiners as to their manner of recording caries, an analysis of variance was carried out (F-test). The diverse number of decayed surfaces per individual and per examiner was studied (Table 6).

TABLE 6. ANALYSIS OF VARIANCE WITH REGARD TO DECAYED SURFACES

Source of variance	Degrees of freedom	Sum of squares	Mean of squares	F
Examiner	9	39·69	4·41	3·2119*
Patients	9	245·29	27·254	19·8502***
Rest	81	111·21	1·373	—
Total	99	396·19	—	—

Vol. 6
1961

The material was derived from Table 2. The F-values in Table 6 show that the differences between the findings of the participating dentists were significant. This implies that the differences were systematical and also that, in a comparison of the average figures, some dentists are "over-recorders" and others "under-recorders".

A similar result is obtained from the analysis of the data in Table 1 (Table 7.).

TABLE 7. ANALYSIS OF VARIANCE WITH REGARD TO DECAYED TEETH

Source of variance	Degrees of freedom	Sum of squares	Mean of squares	F
Examiner	9	31·25	3·4722	3·02246**
Patients	9	222·45	24·7167	21·51523***
Rest	81	93·05	1·1488	—
Total	99	346·75	—	—

In the next analysis of variance (fissure caries, Table 8) the F-value does not, however, reach the level of significance. This does not mean that there were no differences between the findings of the examiners. The low F-value was most likely due to the limited extent of the material.

TABLE 8. ANALYSIS OF VARIANCE WITH REGARD TO FISSURE CARIES

Source of variance	Degrees of freedom	Sum of squares	Mean of squares	F
Examiner	9	4·16	0·46222	1·507
Patients	9	25·96	2·88444	9·40568***
Rest	81	24·84	0·30667	—
Total	99	54·96	—	—

The results presented in Table 5 were also subjected to an analysis of variance (Table 9).

TABLE 9. ANALYSIS OF VARIANCE WITH REGARD TO THE RECORDING OF CARIES ON FULL MOUTH ROENTGENOGRAMS

Source of variance	Degrees of freedom	Sum of squares	Mean of squares	F
Examiner	9	48·1	5·344	1·69
Patients	4	236·6	59·15	18·72***
Rest	36	113·8	3·161	—
Total	49	398·5	—	—

As may be seen in Table 9 the F-value does not reach the level of significance. This is surprising considering the very great deviation in the number of recorded lesions shown in Table 5. A closer study of Table 5 makes it evident, however, that the examiners with the highest total figures do not record top figures for all patients throughout. In other words, they are not systematical "over-recorders". The purpose of the analysis of variance was to establish whether the difference in recording, as carried out by different examiners, was due to systematical error or not.

The large error in caries recording may be attributed to a number of concurrent factors: In the first place it must be realized that there is no foolproof definition of the term clinical caries. The adequacy and efficacy of our technical aids are also of great importance. The human factor constitutes a highly irrational factor. The ability of different examiners to detect or evaluate a carious lesion has been dealt with earlier. Added to the individual difference in recording caries, the fatigue factor may also be of decisive importance.

In appraising the consequences of the differences in recording by different examiners there is reason to make a distinction between two types of investigation:

(1) Descriptive studies, e.g. cross-section studies, the aim of which is to obtain a mean value for a population, for example the number of carious teeth or tooth surfaces per person;

(2) Comparative studies such as comparisons of the development of caries in two different groups, one treated and the control group not treated, or comparison of the caries status in two different races or the two sexes. In such studies the point of interest is the difference between the mean values of the two groups.

If only one examiner is employed in a descriptive study there is the risk that he may be a typical "over-recorded", or the opposite, and the mean value will be quite misleading, systematically too low. The systematical error will obviously not be reduced by increasing the investigated material. An increase would be an advantage only in so far that sampling errors will be reduced.

In a comparative study the results will not be affected to the same extent if the same examiner is used in both groups. An "under-recorder" will of course obtain too low values in both groups but in comparing the values the systematic errors will mainly cancel each other.

If more than one examiner is employed in investigations of the descriptive type the situation is more hopeful. To meet with two typical "over-" or "under-recorders" at the same time is fairly improbable, and there is also the possibility of comparing the mean values obtained by both examiners. A great difference between the mean values would be an indication that at least one of them must be an "over-recorder" or "under-recorder". This argument holds good regardless of whether the examiners have made recordings of the same patient (duplicate recording) or each examiner has recorded every other patient, or made a random selection out of the whole case material.

Vol. 6
1961

There is a certain advantage in employing several examiners in comparative studies as well as in descriptive studies, regardless of the method of recording. It is, however, definitely inadvisable to assign one whole group to each examiner, the treated group to one and the control group to the other. If this were done, the differences between the groups might be attributed to the systematic deviations between the two examiners and not be a definite expression of the effect of the treatment.

In planning clinical investigations of caries there is usually a certain limit to the funds available and it is therefore necessary to consider which measures are the most expedient and possible to carry out.

In a very simple pattern situation it is at least possible to decide on a criterion of successful planning. If the purpose of the investigation is limited to a comparison of the effect of two caries-inhibiting agents with the view only to find out which agent has the greater effect and not how much greater, then the investigation is simply planned for establishing the difference between the mean values for the treated group and the control group. The difference is then subjected to statistical analysis of significance. When the investigation has to be planned within certain financial limits it is of course to the greatest advantage to employ a method of analysis which will most decisively establish significance in preference to a method by which vague or no significance is obtained. The significant differences must be attributable, however, to the effect of treatment and not to the errors of recording. The following example may be given: The purpose of an investigation is to study the effect in children of two caries inhibiting agents. The recording may comprise the entire dentitions, and in order to keep the expenses within the given limits, the investigation must be limited to fifty children in each group. At the same cost one half of the dentition may be recorded in eighty children (partial recording) per group. The correlation between total and partial recording is very high, $r = 0{\cdot}96$ (WELANDER, 1959). This means that if there are any real differences between the caries inhibitors the possibility of securing significance is considerably greater when applying

partial recording, since the number of independent units for analysis is in the ratio of 80/50.

The efficacy of the planning may also depend on other factors. Those that may be considered as possible to vary are:

(1) Number of patients. Increased case material should give a greater possibility of establishing an effect of the treatment;
(2a) Number of examiners taking part;
(2b) Number of examiners per patient (single or duplicate recording);
(3) Number of recorded teeth or surfaces per patient (partial or total recording);
(4) Technique of recording (examination with probe and mirror only or supplemented by X-ray and previous cleaning up of the dentition).

When the costs of the investigation are fixed beforehand these four points will have to be balanced against one another. If each patient is to be recorded in duplicate the number of patients will have to be reduced. If the whole dentition is to be recorded, as apposed to partial recording, it is also necessary to cut down the number of patients. In order to make the most profitable balancing it is necessary to be fully informed of:

(a) The cost of total recording of a patient carried out by one examiner, and the cost of duplicate recording by two examiners;
(b) The cost of partial and total recording of one patient when supplementary X-ray is used;
(c) Correlation between partial and total recording;
(d) Systematic differences between examiners and between purely clinical and clinical-radiographical examination. Disparity between different examiners in recording the same patient groups;
(e) The disparity between patients as regards the prevalent caries variate (so-called biological variation).

These data are required for the calculation of significance. It would take too much time, however, to enter into details here of how these data are used. At present there are unfortunately only isolated reports obtainable of the costs and variations which I have mentioned.

Experience from research in other fields shows that there is a great deal to be gained by devoting greater attention to the actual planning of investigations.

In this connexion the question of record notes should also be touched upon. The sub-committee on standardization of caries recording methods have worked out their proposals in which they recommend that the records are made on a so-called tooth-diagram, and it is pointed out that anatomical diagrams would give the best results. The diagram being anatomically correct would enable the examiner to draw a picture of the actual extent of the cavity. As the examiner himself has to make this drawing and thus cannot rely on an assistant, much time is lost. It may

also be asked which caries problems are so simple that this method of recording is fully satisfactory? In a study of the development of untreated caries it is of course useful to have correct knowledge of the extent of each cavity, but the question is whether it is possible to obtain this knowledge from a drawing of the surface expanse of the cavity without any indication of its depth. Instead of anatomical diagrams the so-called Moulage system would seem to offer greater possibilities, the system being devised for a qualitative as well as a quantitative evalutation of each cavity.

Experience has further proved that in large-scale statistical investigations of carries all modern technical recording aids such as machines for data recording on perforated cards ought to be used in order to save time and ensure accuracy. All record data are transferred to perforated cards, and the filing, tabulation, etc., are performed electrically. For a simple transfer of caries data a dental card should be used on which the records can be entered for each tooth and each surface, the card being in a suitable tabular form. The recording is carried out in the way shown in Fig. 1.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
	M	Cf	F or D	Caries lesions					Filling					Extraction	Exposed tooth neck	Diastema	Hypoplasia	Erosion	Abrasion	Calculus	Pocket formation		Crowding
				mes.	Occl.	dist.	bucc.	cem.	m	o	d	b	c								Severity	Site	
7+	1																						
6+		1																		×			
5+			1					×				×			×								
4+			1		×	×										I							
3+		1																					
2+		1														I							
1+		1																					
+1		1														I				×			
+2			1								×												
+3			1						×														
+4					×																		
+5		1																					
+6			1						gold-crown														
+7																							

FIG. 1.

THE CHOICE OF UNITS FOR RECORDING AND STATISTICAL ANALYSIS OF SIGNIFICANCE

In using the mathematical–statistical equation complex which is used in the elucidation of intricate caries problems it is necessary to consider the premises for those equations. The method of statistical analysis of significance which is most commonly used is based on the presumption that the various units for analysis are independent of each other. This implies that the unit of recording (the tooth or the surface) is not identical with the unit of analysis (the individual person). One consequence of this is that the individual must be chosen as the unit of analysis. In other words, the extent of caries prevalence must be calculated for each individual.

The natural recording unit in clinical studies of caries is the tooth or the surface. When the observations are recorded and put together a description may be made in different ways. We may, for instance, record the number of carious surfaces as a percentage of the total number of existing surfaces or state the quotient of the carious surfaces and the total number of surfaces in the examined group. When going a step further and calculating confidence intervals or carrying out analysis of significance, the problem arises of how many independent units are to be reckoned with. It is accepted as a general rule that the number of independent units does not exceed that of the primary sampling units, namely the number of individuals. This leads to certain practical consequences in the account of the results if confidence intervals are to be calculated, since the unit for analysis is always the individual and not the tooth. The method to follow then will be to record the number of intact teeth per patient or to use one of the accepted caries indices such as DMFT or DMFS. The index is recorded for each patient, whereafter mean values and disparity are calculated for the whole group.

The recordings of 2800 teeth in 100 persons thus means that there are only 100 independent units of recording, not 2800, that is to say the same number of units of recording as if only one tooth had been examined in each person.

It must be borne in mind that unfortunately the accuracy of the caries indices is not what it ought to be, since the indices are influenced by factors such as conditions of previous dental treatment. By way of illustration, let us take a set of identical twins who differ only in the way that dentistry has been applied. In one twin teeth with primary lesions have been extracted while in the other twin the cavities were filled immediately on appearance. If the number of caries-free teeth is recorded as an expression of the caries status of the patient, it may be assumed that the susceptibility of the other teeth will be lower for the twin whose teeth were extracted; in him the tendency for caries-free teeth will be greater than in the other twin whose carious lessions had been replaced by fillings.

From this example it is apparent that the caries indices of either type do not eliminate the differences in dental treatment; the accuracy of the caries index is therefore fictive. Comparisons of the dietary practice in different population groups

indicate that there is no standardized term for denoting dental treatment. Any effect of dietary factors on the dental status may thus be masked by dental treatment. The same applies when the caries status is expressed as the total number of caries-free teeth. In an analytical investigation the caries measure may be influenced by irrelevant factors.

The question of units for analysis and sampling may become a crux in another connexion. Studies of the effect of caries inhibiting agents are commonly carried out on school-children, the material often comprising children from different schools. In the recent investigation of the effect of supervised tooth brushing with a sodium fluoride solution, the control material consisted of children of certain ages from ten schools, all situated in Stockholm, as were the test schools.

Vol. 6
1961

A survey of the percentage of intact teeth showed fairly great variations, and the question presented itself whether these variations were merely chance phenomena or whether the school and school environment were responsible for the caries status of the children from each school. If there is a definite relation between the children of the same school we must use the school and not the child as the unit for statistical analysis, in accordance with our previous conclusions.

The child may be used as the unit if the percentage of caries-free teeth in the ten control schools shows a standard mean deviation of a value compatible with the hypothesis that the children of the ten schools might constitute ten samples of the same population.

The statistical analysis proved that the difference between the schools with regard to the relative rate of caries-free teeth was significant; the difference was actually so pronounced that it could not be considered a mere chance. There seemed to be a similarity between the children of the same school. Thus we had to use the school and not the child as the unit. In studies of this kind it is therefore to be recommended that groups to be compared with each other are selected from the same school.

REFERENCES

BERGGREN, H. and WELANDER, E. 1960a. Supervised tooth brushing with a sodium fluoride solution in 5,000 Swedish school children. *Acta odont. scand.* **18**, 209.

BERGGREN, H. and WELANDER, E. 1960b. The unreliability of caries recording methods. *Acta odont. scand.* **18**, No. 4.

GYTHFELDT, T. 1938. Die Grundlage Cariesstatistische Untersuchungen. *Norske Tandlaegeforen. Tid.* **48**, 243.

LUNDBERG, M. and MARKÉN, K.-E. 1958. En jämförelse mellan bissektrisinställning och bitewingteknik vidr öntgenologisk diagnos av cervico-proximal sekundärkaries. *Svensk tandläk. Tidskr.* **51**, 482.

RADUSCH, D. T. 1941. Variability of diagnosis of incidence of dental caries, *J. Amer. dent. Ass* **28**, 1959.

WELANDER, E. 1955. *The Occurrence of Dental Caries in The Permanent Dentition.* Almqvist and Wiksell, Uppsala.

WELANDER, E. 1959. Partial recording of dental caries. *Acta odont. scand.* **18**, 377.

YULE, G. U. and KENDALL, M. A. 1945. *An Introduction to The Theory of Statistics.* pp.368–369. London.

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 70–79, 1961. Pergamon Press Ltd.

RELATIONSHIP OF OCCLUSION AND ORAL CLEANLINESS WITH CARIES RATES

J. MILLER

Turner Dental School, Bridgeford Street, Manchester 15, England

Abstract—In 700 12-year-old children gingival conditions were related to occlusion; the following levels of healthy gingival conditions were found:

(1) in children with a normal occlusion 27 per cent had healthy gingivae,

(2) in children with a malocclusion 14 per cent had healthy gingivae.

In the same group of children regular toothbrushing—at least once per day—was associated with better gingival conditions in children with either a normal or abnormal occlusion. Average DMF rates were found to be higher with regular toothbrushing than with irregular or no toothbrushing—the difference gave a figure for *P* less than 0·05. Relating occlusion to caries children with a malocclusion had higher DMF rates than children with a normal occlusion—the difference was not significant at age 12 years; but comparison of caries rates and occlusion in 268 children aged 14 years showed that 157 children with normal occlusion had an average DMF of 8·27 and 113 with a malocclusion had an average DMF of 9·49; this difference was more than three times the standard error.

Zusammenfassung—Bei 700 12 Jahre alten Kindern wurde der Gingivazustand zum Biß in Beziehung gebracht. Es wurden folgende Verhältnisse gefunden:

1. 27 Prozent der Kinder mit normalem Biß hatten gesunde Gingivae.
2. 14 Prozent der Kinder mit anormalem Biß hatten gesunde Gingivae.

Bei der selben Kindergruppe war regelmäßiges Zähneputzen (wenigstens einmal pro Tag) mit gesünderen Gingivae verbunden, und zwar sowohl bei den Kindern mit normalem Biß wie bei denen mit anormalem Biß. Die durchschnittlichen DMF-Werte waren bei regelmäßigem Zähneputzen höher als bei unregelmäßigem oder keinem Zähneputzen. Der Unterschied ergab eine Zahl für *P*, die unter 0,05 lag. Wenn man die Bißstellung zur Karies in Beziehung setzt, so hatten Kinder mit anormalen Biß höhere DMF-Werte als Kinder mit normalen Biß. Der Unterschied war im Alter von 12 Jahren nicht signifikant, aber der Vergleich von Kariesbefall und Biß bei 268 Kindern im Alter von 14 zeigte, daß 157 Kinder mit normalen Biß einen durchschnittlichen DMF-Wert von 8,27 hatten und 113 mit anormalen Biß einen durchschnittlichen DMF-Wert von 9,49. Dieser Unterschied übertrifft die Standardabweichung um mehr als das dreifache.

INTRODUCTION

MANY clinical surveys have confined themselves to only one aspect of dental disease; some have reported on more than one dental condition but few considered the interrelationship of dental conditions. It has been shown (MILLER, 1960) how comparisons of caries rates could be misleading when they were based solely on

DMF rates and did not consider the number of erupted teeth. Fig. 1 shows the average DMF in the first permanent molar of 6 year old children between 1950 and 1958. It would appear from this that the average DMF has fluctuated greatly and reached a level in 1958 almost twice that in 1950. If we relate this average DMF

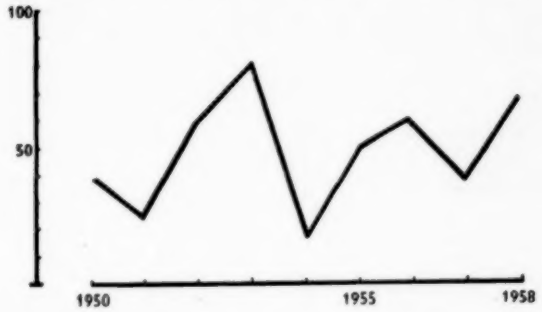


FIG. 1. Average DMF per 100 children aged 6 years (first permanent molars only).

to the percentage of first permanent molars that are erupted (Fig. 2, line b) it is seen that the DMF is similar to the percentage of teeth erupted. If from these is found the percentage of erupted first permanent molars that are carious (Fig. 2, line c) it will be seen that there is much less fluctuation in caries rates—the assess-

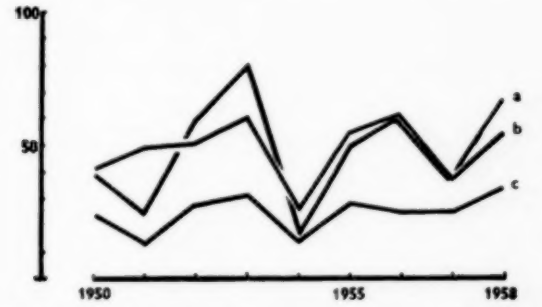


FIG. 2. Relationship between Average DMF, eruption of the teeth and percentage of teeth carious at age 6 years (in first permanent molars): a = Average DMF; b = Percentage of first permanent molars erupted; c = Percentage of erupted first permanent molars that were carious.

ment made solely on average DMF so reflects more the change in the eruption of teeth than a change in the caries attack. Treatments which could influence the eruption of teeth could thereby influence the carious attack and lead to false conclusions of the effect of the treatment.

In this report it will be shown how the occlusion may influence not only the incidence of gingival conditions but also caries rates; also the effect of oral cleanliness on both gingival conditions and on dental caries will be shown.

Vol. 6
1961

These findings are based on more than 10 years continuous observation of the pupils of three Manchester schools, aged 3–15 years and numbering at any one time 1200. The schools were situated in a poor area of Manchester and few children had received any dental treatment before attending.

STANDARDS OF DIAGNOSIS

General

All examinations were made in the dental school; the patient being in a dental chair and the mouth illuminated by Anglepoise lamp of not less than 60 W. The teeth were examined dry after previous complete cleaning by a hygienist.

Malocclusion

Malocclusion was diagnosed and classified according to ANGLE (1907) and the results are presented to show the incidence of:

Normal occlusion
Class I
Class II } Malocclusion
Class III

Caries

All examinations were made with a replaceable probe (MILLER and ATKINSON, 1951), each replaceable probe point being used for not more than seven examinations. The minimum lesion which was recorded as caries was:

(1) In pits and fissures—when the probe stuck in a fissure and required a definite pull for removal;
(2) In proximal surfaces—when by means of the probe Ash 12, a break in the surface of a tooth was detected.

Examinations were made once every 6 months and followed a set routine of procedure (JACKSON, 1950). The examinations were arranged to coincide with the month of the child's birthday to reduce age variation.

Gingival conditions

The gingival condition was assessed as:
Good—normal healthy pink mucosa with stippling;
Fair—loss of stippling—marginal redness of the gingivae;
Poor—acute or chronic inflammation regardless of extent or site.

Oral cleanliness

The children were brought in for examination direct from the schools by a member of the hospital staff. The children never had prior warning that they might be examined on any particular day—it was possible by this means to examine the standard of oral hygiene that prevailed normally. Prior warning of a dental appoint-

ment often stimulates a patient to a standard of oral hygiene temporarily higher than that which pertains during most of the inter-appointment period. The standard of oral cleanliness was assessed by questioning the patient and by the evidence of the intraoral examination before routine cleaning; if the reply from the patient indicated a more regular oral cleanliness regime than the appearance of the teeth substantiated, then the evidence of the intraoral examination was preferred. Oral cleanliness was classified:

Regular—toothbrushing once a day—clean teeth without attached food debris;
Irregular—toothbrushing less than once a day;
None—no toothbrushing.

During the 10 years a total of 5000 different children have been examined and over 20,000 examinations made.

Vol. 6
1961

Significance

A difference between two findings has been considered significant when the difference between the mean or proportions was more than two and a half times the standard error or when in χ-square test the figure for P was less than 0·05.

FINDINGS

Caries

Though this report is concerned primarily with the interrelationship of dental conditions, background information is required of the level of dental disease present in the children under study. A brief description of the carious pattern in these children is given here.

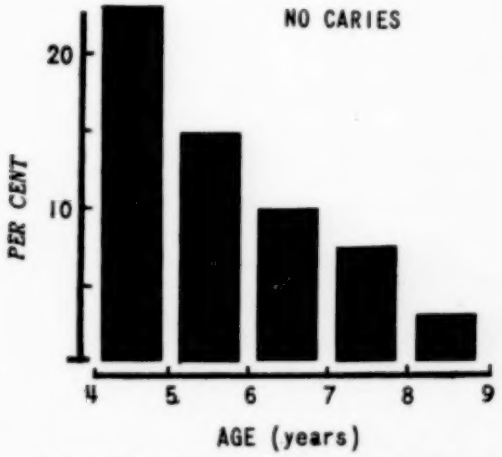


FIG. 3. Caries-free children (deciduous teeth).

In the deciduous teeth, the percentage of caries free children ranged from 24 per cent at age 4 years to 3 per cent at age 8 years (Fig. 3). The most caries-prone

teeth were the deciduous molars (Fig. 4). At the age of 4 years more caries was found in the occlusal than in the approximal surfaces of the deciduous molars, but by age 5 years more was found in the approximal surfaces (Fig. 5).

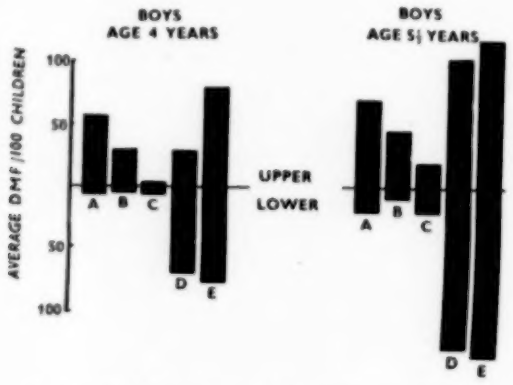


FIG. 4. Incidence of caries in deciduous teeth.

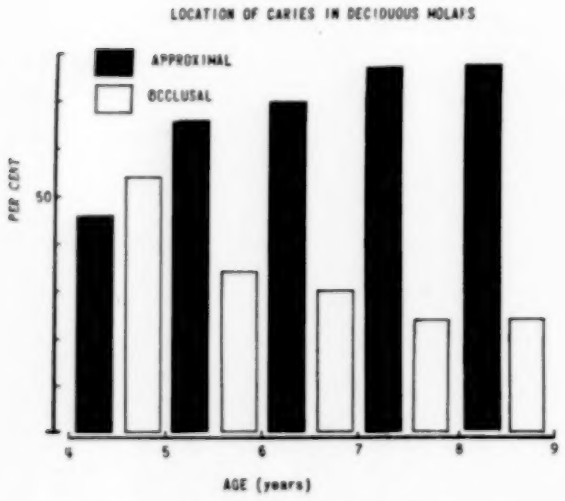


FIG. 5. Location of caries in deciduous molars.

Gingival conditions

It was found in examining the children that the gingival condition deteriorated from age 7 to 12 years, during the period of the mixed dentition, (MILLER, 1950), and this age period was not a satisfactory one from which to draw conclusions. Studies of the gingival conditions were therefore made on 775 12-year-old children. Good

gingival conditions were found in 26 per cent of the girls and in only 17 per cent of the boys (Table 1). When gingival conditions were related to occlusion (Table 2) it was found that an abnormal occlusion was associated with poorer gingival

TABLE 1. PERCENTAGE OF GINGIVAL CONDITIONS AT AGE 12 YEARS

	Gingival conditions (%)			No. of children
	Good	Fair	Poor	
Boys	17	49	34	369
Girls	26	50	24	406

Vol. 6
1961

TABLE 2. GINGIVAL CONDITIONS RELATED TO OCCLUSION AT AGE 12 YEARS

Occlusion	Gingival conditions (%)			No. of children
	Good	Fair	Poor	
Normal	27	48	25	426
Abnormal	14	52	34	349

condition; good gingival conditions were found in 27 per cent normal occlusion but in only 14 per cent of abnormal. This difference in gingival conditions between boys and girls was not due to the occlusion for the incidence of normal and

TABLE 3. OCCLUSION IN 369 BOYS AND 409 GIRLS AGED 12 YEARS

Occlusion	Boys (%)	Girls (%)
Normal	55	55
Abnormal	45	45

abnormal occlusion was the same in girls and boys (Table 3). The better gingival condition of girls was possibly due to better oral hygiene; for 20 per cent of the

TABLE 4. ORAL CLEANLINESS AND GINGIVAL CONDITIONS AT AGE 12 YEARS

Oral cleanliness	Gingival conditions		
	Good (%)	Fair (%)	Poor (%)
Regular	58	37	5
Irregular	31	52	17
None	12	49	39

girls showed regular oral hygiene compared with 13 per cent of the boys. Regular oral cleanliness was associated with better gingival conditions (Table 4).

Oral cleanliness and caries rates

The relationship between oral cleanliness and DMF rates was analysed on 736 12-year-old children (Table 5). Many of these children were the same as those in the above findings but they were not identical in every individual. The findings

TABLE 5. RELATIONSHIP OF ORAL CLEANLINESS AND DMF RATES AT AGE 12 YEARS

	Oral cleanliness		
	Regular	Irregular	None
No. of children	115	264	357
Av. DMF at age 12 years	5·8	5·0	5·0

are presented to show the average DMF rates for boys and girls combined, not distinguishing between normal and abnormal occlusion. These factors were studied separately but did not add greatly to the information when presented separately. This showed a higher DMF rate with regular oral cleanliness than with no oral cleanliness; this difference was statistically significant.

Occlusion and caries rates

An abnormal occlusion usually results in an increase in the number of stagnation areas in a mouth; also the abnormal occlusion often results in less efficient cleaning of the teeth by the passage of food over them and by the action of the

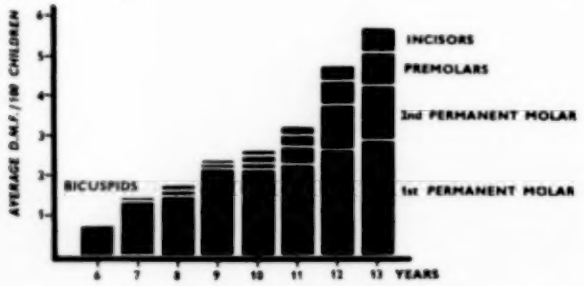


FIG. 6. Incidence of caries in permanent teeth.

lips and cheeks. Dental caries is usually associated with areas of stagnation. It is possible that an abnormal occlusion causes a higher caries rate by reason of an increase in the number of stagnation areas. This possibility was examined. At age 12 years no significant difference was found between the DMF rates of children with a normal occlusion and those of children with an abnormal occlusion. In

children aged 14 years the difference was significant—157 children with a normal occlusion had average DMF 8·27, 113 children with an abnormal occlusion had

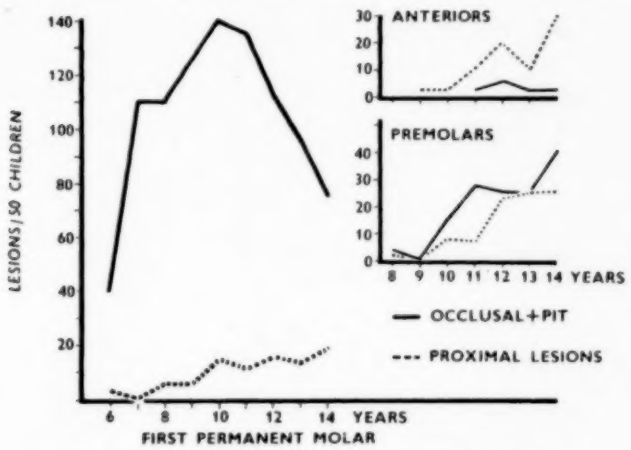


FIG. 7. Location of caries in permanent teeth.

TABLE 6. RELATIONSHIP OF OCCLUSION AND DMF

Occlusion	Average DMF	No. of children
Normal	8·27	157
Abnormal	9·49	113

average DMF 9·49 (Table 6). It was concluded that a malocclusion was associated with a higher incidence of caries.

DISCUSSION

That a significant association is shown to be present between two conditions does not indicate cause and effect between those conditions. It may be that cause and effect may later be proven but until so, care should be taken not to interpret the findings incorrectly. It is rarely possible in a clinical study to take into consideration all possible variables at one and the same time; that associations have been demonstrated between some of the conditions examined does not exclude the possibility of other influencing factors. In a study of this nature it will be appreciated that the assessment of oral cleanliness related more to an objective assessment of the appearance of the mouth than to efficacy of tooth brushing, for the latter could vary greatly from brushing once a day to brushing after every meal. Similarly it did not distinguish between tooth brushing before breakfast and tooth brushing

after the evening meal. The assessment was of the standard of oral cleanliness at examination of a child to whom no warning had been given of intention to examine.

The findings relating an association between oral cleanliness and gingival conditions are not surprising. It is the findings relating the association of higher DMF rates to the cleaner mouths that are surprising. This could not be discounted on the basis that in the mouths with no oral cleanliness the food debris obscured the caries, for, after the assessment of oral cleanliness and before any diagnosis of caries, all teeth were cleaned and polished by hygienists.

Few studies have compared the effect of average tooth brushing with no tooth brushing; most studies have compared the effect of rigorous tooth brushing with average tooth brushing. MANSBRIDGE (1960) found an association between oral hygiene and reduced caries rates; it is interesting and it may also be significant in MANSBRIDGE's findings that not only was the percentage of children performing satisfactory oral hygiene twice that in the present study but also the caries rates were twice as high. It would appear that tooth brushing as practised in the present group of children did not reduce the incidence of caries in school children under the age of 12 years, but did improve the gingival condition—comparable findings were reported by HINE (1948) and HEIN (1954).

These findings may indicate that oral hygiene may be more effective against a certain type of caries. In the present study the caries was mainly in the pits and fissures. It may be that oral hygiene is more effective against smooth surface caries or that its effect may be more demonstrable against high DMF rates. The present findings on raised DMF rates with regular oral cleanliness require confirmation; with corroborative evidence they will indicate that a reappraisal should be made of dental advice. Tooth brushing is beneficial for gingival conditions but is it of equal caries prevention value for 3-year-old patients and for 60-year-old patients? It may be that we should design our advice more specifically so that the same panacea of tooth brushing is not prescribed for all ages of patients and in the same form of application for all dental conditions of man.

That DMF rates are higher with an abnormal occlusion indicates the need for assessments of occlusion in surveys of dental caries. In the same way as groups of children may differ in the proportions of approximal and occlusal caries so may they in the incidence of abnormal occlusion. Ideally, studies of caries should be made on children with normal occlusion only, but such an ideal is rarely possible—hence the need to assess the proportion of abnormal occlusion present in the population under study. In the present findings the differences in DMF rates were not "significantly" different at age 12 years, but were at age 14 years. It is possible that at ages 16, 18 and 21 years these differences would be progressively more evident. These findings indicate that in order to prevent aggravation both of the caries problem and of the gingival problem it is necessary to prevent malocclusion. The findings merely draw together in association several factors which are present in

children's mouths; it is often necessary to assess and treat each separately, but they should be related to one another and to the other factors when planning treatment and when prevention in dentistry is being practised.

REFERENCES

ANGLE, E. H. 1907. *Treatment of Malocclusion of the Teeth* (7th ed.) S. S. White Dental Manufacturing Co., Philadelphia.

HEIN, J. W. 1954. *J. dent. Res.* **33**, 708.

HINE, M. K. 1948. *J. dent. Res.* **27**, 170.

JACKSON, D. 1950. *Brit. dent. J.* **88**, 207.

MANSBRIDGE, J. N. 1960. *Brit. dent. J.* **109**, 343.

MILLER, J. 1950. *Dent. Practit.* **1**, 66.

MILLER, J. 1960. *J. dent. Res.* **39**, 1111.

MILLER, J. and ATKINSON, H. F. 1951. *Brit. dent. J.* **90**, 157.

Vol. 6
1961

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 80–93, 1961. Pergamon Press Ltd.

AN EPIDEMIOLOGICAL STUDY OF DENTAL CARIES PREVALENCE IN ADULTS

D. JACKSON

Dental School and Hospital, University of Leeds, England

Abstract—As age increases, the DMF index becomes progressively less sensitive as a measure of caries prevalence. The assumption that all missing teeth were at one time carious is not valid in adults. If the DMF index is to be used in adults, it is necessary to apply a correction factor for each type of tooth and for each age. In order to facilitate the use of the DMF index beyond childhood, correction factors were determined by collecting all teeth extracted by sixty dental practitioners during a specified period and assessing the number which were carious, the reason for extraction being immaterial. These correction factors were applied to data obtained from an epidemiological study of caries prevalence in 1441 adults aged 15–64 years. It was found that the DMF index for all teeth could be used up to the age of 25 years with small error, but beyond this age it was essential to use correction factors. It was found, however, that caries prevalence rates in adults could be satisfactorily assessed by confining the DMF rates to molar teeth (excluding third molars) and upper premolar teeth without correction, the percentage number of those extracted which were carious, being extremely high. Whereas the correction factors must apply specifically to the community studied, the method of approach which has been described has universal applicability.

Zusammenfassung—Mit zunehmendem Alter wird der DMF-Index als Maß für das Vorhandensein von Karies immer unempfindlicher. Die Annahme, daß alle fehlenden Zähne früher kariös waren, gilt nicht bei Erwachsenen. Wenn der DMF-Index bei Erwachsenen benutzt werden soll, ist es notwendig, einen Korrekturfaktor für jeden Zahntyp und für jedes Alter anzuwenden. Um die Anwendung des DMF-Index jenseits des Kindesalters zu erleichtern, wurden Korrekturfaktoren bestimmt durch Sammeln aller Zähne, die in einer bestimmten Zeit von 60 praktischen Zahnärzten extrahiert wurden und deren Kariesbefall festgestellt wurde. Die Indikation zur Extraktion blieb dabei unberücksichtigt. Diese Korrekturfaktoren wurden bei der Auswertung einer epidemiologischen Untersuchung über das Kariesvorkommen bei 1441 Erwachsenen im Alter von 15–64 Jahren angewandt. Es zeigte sich, daß der DMF-Index für alle Zähne bis zum Alter von 25 mit kleiner Fehlerbreite benutzt werden konnte, über dieses Alter hinaus aber war es wesentlich, die Korrekturfaktoren zu verwenden. Es wurde aber gefunden, daß das Kariesvorkommen bei Erwachsenen zufriedenstellend festgestellt werden konnte, wenn man den DMF-Index auf die Molaren (außer den 3. Molaren) und die oberen Prämolaren ohne Korrektur beschränkte, da der Prozentsatz des Kariesbefalls bei diesen Extraktionen äußerst hoch waren. Obwohl die berichteten Korrekturfaktoren nur für den beobachteten Personenkreis gelten, kommt der Auswertungsmethode allgemeine Bedeutung zu.

Vol. 6
1961

INTRODUCTION

THE PROBLEM as to whether the beneficial effects of fluoridation, or indeed any other method for preventing dental caries are permanent or whether they decrease with age has not been satisfactorily determined. One major difficulty is the accurate assessment of caries prevalence in adults. Whereas the DMF index is universally accepted as the most useful and practical index of caries prevalence in children, the index becomes progressively less accurate as age advances. This is because the basic assumption that all missing teeth were at one time carious, whilst valid in children, becomes increasingly unjustifiable as more and more teeth are extracted for other reasons than caries. RUSSELL and ELVOVE (1951) attempted to compensate for this inaccuracy in adults by recording the primary reason for extraction based upon a history of signs and symptoms. Apart from the fact that subjective evidence of this nature is unreliable a tooth removed for any reason other than dental caries may, at the same time, have suffered caries attack. Unless reliable life records are available, an accurate assessment of the cumulative incidence of dental caries over a wide age spectrum would seem to be impossible. On the other hand, if by a sampling survey, the percentage number of extracted teeth which were or had been carious was known for any specific community, it would appear reasonable to translate these findings to the observed DMF prevalence rates of that particular community. In order to test the practicability of this approach the present investigation was commenced.

INVESTIGATION

1. *Epidemiological study*

The first part of the investigation was an epidemiological study of 1441 adults aged 15–64 years who were employees of two factories in Leeds (F=0·06 p.p.m.). In order to study as large an age spectrum as possible, similar and contemporaneous data pertaining to 1040 12-year-old Leeds children are also presented. Dental caries was diagnosed according to the procedure described by JACKSON (1950) and SLACK *et al.* (1958). From the data so obtained, DMF prevalence rates were obtained for each tooth type (excluding third molars) at each age level studied.

2. *DMF correction*

The second part of the investigation was designed to determine the proportion of carious to non-carious teeth in those extracted in order to correct the observed DMF values. It consisted of collecting, during a specified period of time, all teeth extracted by sixty dental practitioners from the same community in which the epidemiological study was carried out. The teeth were separated according to age and type, cleaned and examined for dental caries. A probe was used if caries was not frank or obvious to the naked eye: all filled teeth were included in this category.

It was thus possible to determine, according to age and tooth type, the proportion of extracted teeth which had been attacked by dental caries. Third molars were not considered in this investigation because of their variability.

FINDINGS

In order to present the observed and corrected DMF values together, it is necessary to report the findings of the second part of the investigation first.

1. *DMF correction* (*Table* 1)

It was anticipated that of those teeth extracted, the ratio of sound to carious teeth would vary from age to age. Contrary to this expectation, the ratio for any particular tooth type was virtually constant for all ages above 15 years. At 12 years, however, there was a difference. Whereas at this age 75 per cent of extracted upper premolar teeth were caries-free, the corresponding figure for all the other teeth was only 1 per cent. There was, therefore, a minor correction necessary at 12 years, but since the proportion of missing premolar teeth was relatively small, this correction made no material difference to the observed DMF rates. The teeth were collected in age groups of 5 years, such as 15–19 years, 20–24 years, up to 64 years and the correction factors applied to the DMF values pertaining to these age groupings. The rates did not vary significantly with age and hence may be taken to be constant. The percentage number of sound teeth for each tooth type are given in Table 1. The choice of tooth types was determined by analogous susceptibilities to caries attack, reference to which will be made later.

TABLE 1. THE NUMBER OF EXAMINED EXTRACTED TEETH AND THE PERCENTAGE NUMBER OF TEETH FOUND TO BE CARIES-FREE IN ADULTS FROM 15 TO 62 YEARS

	No. of teeth	Caries-free (%)	Carious (%)
Upper incisors	234	35	65
Canines	352	52	48
Upper premolars	282	13	87
Molars	701	3	97
Lower incisors	299	79	21
Lower first premolars	176	41	59
Lower second premolars	195	27	73

Each tooth within each tooth type grouping had almost identical ratios with respect to the percentage number of extracted teeth which were carious. Thus the correction factor for upper central incisors was the same as that for upper lateral incisors.

The DMF values were corrected by substituting the observed M fraction by another value corresponding to the calculated number of missing teeth which had been attacked by dental caries. Thus at 12 years the M value for upper premolar teeth was reduced by 75 per cent. Between 12 and 17 years (i.e. the mean age of the 15–19 year old group) the observed increment in M teeth was reduced by 13 per cent, this being the percentage number of sound teeth. This correction of 13 per cent for premolar teeth was henceforward used for all age groups. Correspondingly the M fraction for all other tooth types was corrected. By combining the corrected DMF values of each tooth type, it was possible to obtain corrected DMF values for all teeth.

Vol. 6
1961

2. *Observed and Corrected DMF Values*

(*a*) *Crude DMF prevalence rates for all teeth*

The observed and corrected DMF values for all teeth for each 5 year period from 12 to 62 years are given in Table 2 and graphically presented in Fig. 1. Both values are close and parallel up to an age approximating to 25 years after which

TABLE 2. OBSERVED AND CORRECTED DMF PREVALENCE RATES EXPRESSED AS PERCENTAGE TEETH

Age		No. examined	Observed	Corrected	Difference
Range	Mean				
12	12	1040	23·8	22·9	0·9
15–19	17	394	37·0	36·3	0·7
20–24	22	252	43·3	42·2	1·1
25–29	27	115	49·4	46·1	3·3
30–34	32	127	61·3	53·9	7·4
35–39	37	157	64·5	55·1	9·4
40–44	42	119	69·2	56·6	12·6
45–49	47	94	74·2	58·6	15·6
50–54	52	80	85·3	62·9	22·4
55–59	57	66	89·3	65·0	24·3
60–64	62	37	90·3	65·9	24·4

age these two values diverge at an ever increasing rate. The absolute difference is approximately 1 per cent up to and at 25 years, whereas at the mean age of 62 years it is 24·4 per cent. It is clear that the observed DMF values can be used with confidence up to this critical age of 25 years, and that projection beyond this age is only possible if some correction is made. The essential difference between the observed and corrected DMF values is the number of extracted sound teeth and it is apparent that 25 years is the age at which there is a marked increase in the rate at which sound teeth are extracted.

The corrected DMF values increase at an ever decreasing rate up to the mean age of 47 years, when there is a slight increase to a level which virtually is henceforward only slight. The rate of increase is greatest between 12 and 17 years when the average absolute increase is 2·6 per cent per year. Between 17 and 32 years the

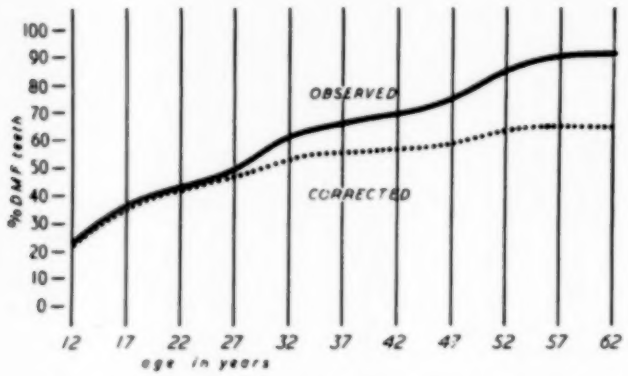


FIG. 1. Observed and corrected DMF values for all teeth.

corresponding value is 1·2 per cent and between 32 and 47 years it is 0·3 per cent. The progressive diminution in the rate at which teeth are attacked by dental caries between 12 and 47 years might be explained on the basis of relative susceptibilities in that the more susceptible teeth are attacked more rapidly, thus leaving an ever increasing proportion of those most resistant to attack.

Another possibility is that as more and more sound teeth are extracted there remain fewer teeth available for attack. An attempt to correct for this factor by assessing the chances of a sound tooth to decay during a 5 year period, transferring these chances to the sound teeth extracted during this period and then adding this value to the DMF values, did not, however, materially alter the order or nature of the age prevalence curve.

Whereas both these possibilities are tenable in explaining the progressive diminution in the rate of caries attack such do not constitute a complete explanation. The values for all these are compounded from values pertaining to several different individual tooth types with different susceptibilities and different patterns of age-prevalence curves. It is essential, therefore, to consider the age-prevalence curves for each of the previously mentioned tooth types.

(b) *Crude* DMF *rates for individual tooth types*

DMF values, both observed and corrected, have been plotted for different tooth types, the choice of tooth type being made on the basis of morphology and also on caries susceptibility. Thus the incisors form one morphological tooth type, but

because of the marked difference in caries susceptibilities between maxillary and mandibular incisors, these have to be considered separately. Similarly the premolars form another group, but whereas both maxillary premolars behave identically with respect to caries attack, they behave differently from the mandibular first and second premolars which in turn have different susceptibilities. Thus one must consider these three types separately. Apart from an initial difference in prevalence rates, influenced largely by differences in eruption times, the first and second permanent molars have a similar susceptibility to caries attack and hence these teeth may be combined.

The prevalence rates in each of these groups for each 5 year period from 12 to 62 years will now be presented.

Vol. 6
1961

TABLE 3. OBSERVED AND CORRECTED DMF VALUES FOR EACH TOOTH TYPE DMF EXPRESSED AS PER 100 ERUPTED TEETH

Mean age	Upper incisors		Lower incisors		Canines		Upper premolars		Lower first premolar		Lower second premolar		First and second perm.molar	
	O	C	O	C	O	C	O	C	O	C	O	C	O	C
12	11·5	11·5	1·7	1·7	0·5	0·5	16·7	15·8	4·1	4·1	11·3	11·3	65·3	65·3
17	14·2	13·4	1·1	0·6	2·4	2·3	38·7	37·2	13·3	12·3	33·2	30·7	89·7	89·7
22	25·5	24·1	1·9	1·2	7·4	6·6	51·6	49·6	24·0	22·6	49·0	44·6	89·9	89·9
27	36·7	32·4	9·1	3·4	16·7	12·4	59·8	56·7	33·0	28·7	56·1	49·1	89·5	89·5
32	50·2	40·5	22·6	6·7	31·4	20·3	74·8	69·8	48·8	39·8	68·5	57·8	95·6	95·6
37	57·2	43·3	23·6	6·7	37·9	20·6	79·3	74·5	56·7	44·3	73·2	59·8	94·3	94·3
42	61·9	44·3	33·2	6·9	48·5	28·4	82·7	75·4	57·5	42·0	76·0	58·8	95·5	95·5
47	67·5	47·3	46·0	10·6	58·2	32·9	85·1	76·3	63·8	43·6	77·6	59·5	96·0	96·0
52	82·8	56·2	69·1	12·8	75·9	39·7	88·7	78·7	82·5	53·1	89·3	65·6	97·2	97·2
57	87·1	57·9	75·7	14·0	82·2	42·4	94·7	83·7	84·8	53·0	94·7	69·7	97·9	97·9
62	92·6	60·8	73·6	14·8	85·8	43·9	94·6	84·5	83·8	52·7	91·9	66·2	98·9	98·9

(i) *Upper incisor teeth* (Table 3, Fig. 2). The observed and corrected DMF values are close and parallel up to an age approximating to 25 years, after which they diverge by an ever increasing amount. The general pattern of the age-prevalence curve is similar to that for all teeth combined, in that there is a relatively quiescent period between 32 and 47 years after which there is a slight increase. The curves differ in that the peak velocity occurs between 22 and 32 years in incisor teeth, whereas for all teeth combined it is between 12 and 17 years.

(ii) *Lower incisor teeth* (Table 3, Fig. 3). Of all teeth the lower incisors are least attacked by dental caries and hence possess relatively low prevalence rates. Very few of the lower incisor teeth were filled and the DMF values were mainly composed of the D and M fractions. It has already been stated that 79 per cent of all extracted lower incisor teeth were caries free, and it is no surprise, therefore, that observed

and corrected DMF values diverge to a considerable extent from the time at which there is a marked increase in the rate of extracted sound teeth: this age is approximately 25 years. Although at a much lower level, the general pattern of the age pre-

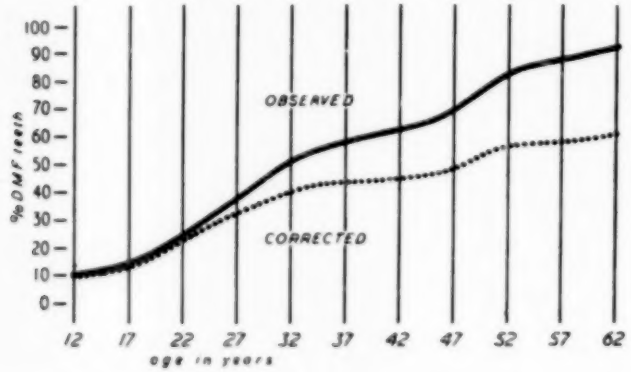


FIG. 2. Observed and corrected DMF values for upper incisor teeth.

valence curve is very similar to that for the upper incisors in that there is a relatively quiescent period between 32 and 47 years followed by a slight increase. It is interesting that the corrected DMF value at 62 years is only 14·8 per cent, whereas it is 60·8 per cent for the upper incisor teeth.

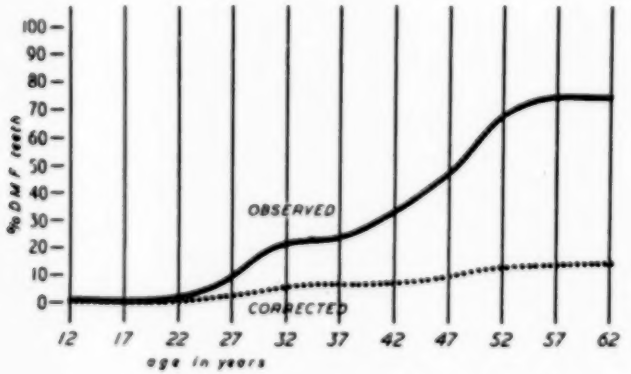


FIG. 3. Observed and corrected DMF values for lower incisor teeth.

(iii) *Canine teeth* (Table 3, Fig. 4). The observed and corrected DMF values are again close and parallel up to an age approximating to 25 years, after which the values diverge by an ever increasing amount. The corrected DMF values increase

at a progressively increasing rate up to the age of 32 years when there is a short quiescent period up to 37 years whence the values increase at a progressively decreasing rate reaching a plateau at 57 years.

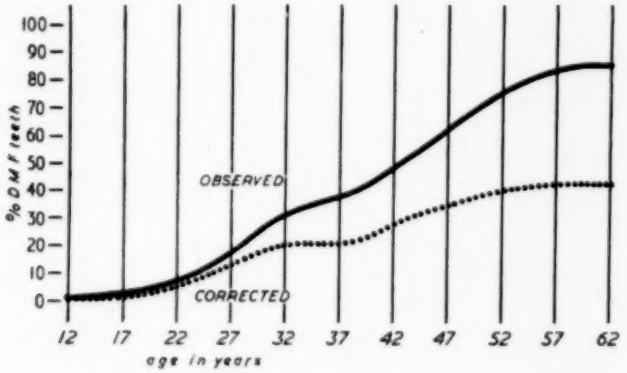


FIG. 4. Observed and corrected DMF values for canine teeth.

(iv) *Upper premolar teeth* (Table 3, Fig. 5). The age-prevalence curve for upper premolar teeth presents a different pattern, both for the observed and corrected DMF values. Both values increase at an ever decreasing rate up to about 37 years. The corrected value at 37 years was 74·5 per cent and at 52 years it was 78·7 per cent

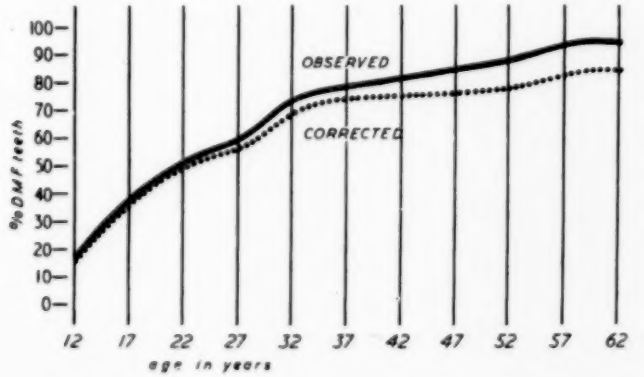


FIG. 5. Observed and corrected DMF values for upper premolar teeth.

so that in this period of 15 years the absolute increase in DMF teeth was only 4·2 per cent. From 52 to 62 years there is a relatively marked increase in the values. The observed and corrected DMF values are close and parallel up to approximately

25 years (absolute difference being 2 per cent) from which age the values diverge by an increasing amount. The maximum rate of increase occurred between 12 and 17 years.

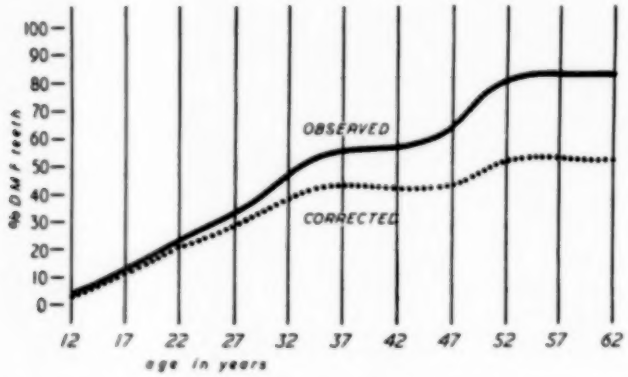


FIG. 6. Observed and corrected DMF values for lower first premolar teeth.

(v) *Lower first premolar teeth* (Table 3, Fig. 6). The age-prevalence curve is different from that for other premolar teeth but very similar to that for upper incisor teeth both in type and also in degree. The significant features are, therefore, the

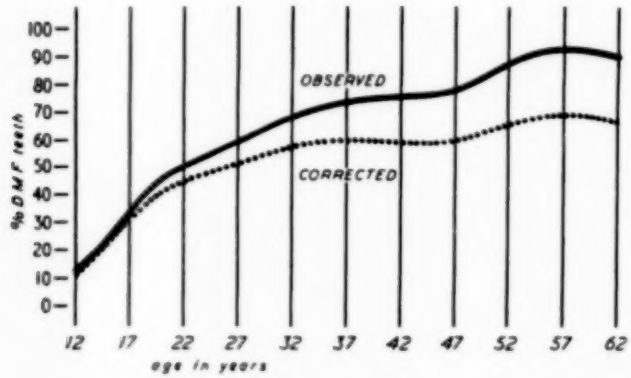


FIG. 7. Observed and corrected DMF values for lower second premolar teeth.

same. The observed and corrected DMF values are close and parallel up to 25 years, and between 32 and 47 years there is a marked quiescent period.

(vi) *Lower second premolar teeth* (Table 3, Fig. 7). The age-prevalence curve is very similar to that for the upper premolar teeth in that up to 47 years the DMF values increase at an ever decreasing rate. Further there is a quiescent period from approximately 32 to 47 years. The observed and corrected DMF values whilst close and parallel up to 17 years diverge progressively henceforward. At 25 years the absolute difference between observed and corrected DMF values is approximately 4 per cent.

(vii) *Molar teeth* (excluding third molars) (Table 3, Fig. 8). For molar teeth the observed and corrected DMF values are virtually identical and hence the observed figures apply to tooth values. The age-prevalence curve for molar teeth is different from that of other teeth mainly because of the exceptionally high susceptibility of

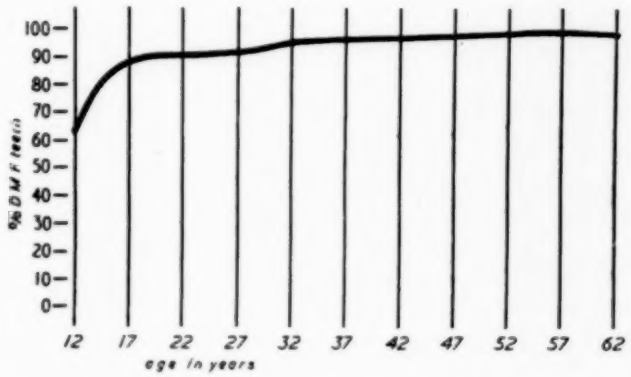


FIG. 8. Observed DMF values for molar teeth.

these teeth. At 12 years 65·3 per cent of all molar teeth were found to have been attacked by caries and at the age of 17 years this figure is just below 90 per cent. Between 17 and 27 years there is a relatively quiescent period, but from 27 years to 62 years there is a gradual increase so that at the latter age 98 per cent had been attacked.

3. *The Use of Observed DMF Prevalence Rates in Upper Premolar and Molar Teeth as a General Measure of Caries Prevalence* (Table 4)

The very high proportion of extracted molar and premolar teeth which were found to have been attacked by dental caries, indicates a very small difference between the observed and corrected DMF values. This suggests the possible use of those teeth as an indication of caries prevalence rates without correction.

In Table 4 are given the observed and corrected DMF prevalence rates for these teeth combined between 12 and 62 years. At 12 years the absolute difference is 0·3 per cent and at the average age of 62 years it is 3·4 per cent.

TABLE 4. OBSERVED AND CORRECTED DMF RATES FOR UPPER PREMOLAR AND MOLAR TEETH COMBINED

Mean age	Observed	Corrected	Difference
12	54·3	54·0	0·3
17	72·7	72·2	0·5
22	77·2	76·5	0·7
27	79·6	78·6	1·0
32	88·7	87·1	1·6
37	89·3	87·3	2·0
42	91·2	88·7	2·5
47	92·4	89·5	2·9
52	94·4	91·1	3·3
57	96·8	93·1	3·7
62	97·5	94·1	3·4

DISCUSSION

Accurate knowledge of caries prevalence in adults is not readily obtained unless accurate dental histories have been recorded. Since the possibility of obtaining such histories in sufficient numbers and over a sufficiently wide age spectrum is virtually nil, then the best one can ever hope to achieve is an approximation. The basic difficulty is that of assessing how many missing teeth, regardless of the reason for their extraction, were attacked by caries. In this investigation a direct approach to the problem has been made by finding out the proportion of extracted teeth which had been so attacked. From this information the number of M teeth in the DMF index was corrected for each individual tooth type according to age. It was thus possible to obtain a corrected DMF value at each age level and for each tooth type.

This method, however, cannot be taken to give an absolutely correct assessment of caries prevalence because it presumes that the proportion of extracted teeth which were carious was constant throughout the life of those examined. There are indeed many conceivable factors which would influence this proportion and because of this it was of considerable interest to find that after the age of 15 years the current proportion was markedly constant for each tooth type. Another difficulty associated with this problem is that extracted teeth reduce potential attack. An attempt to assess potential attack was made by calculating the chances of attack over a 5 year period of the remaining sound teeth at the beginning of this period and converting

this to those extracted during this time. The increase in DMF values over and above the corrected DMF values was so small as to be negligible.

Measurement is never absolute and need only possess an accuracy sufficient for the practical need. The DMF index is not accurate in the absolute sense because of the difficulties associated with the clinical diagnosis of early lesions, and also because of the assumption that all missing teeth were at one time carious. In children errors of diagnosis are likely to be greater than those involved in the assumption, whereas in adults the reverse is likely to be the case. Nevertheless, providing careful standards of diagnosis are employed, the DMF index gives an accuracy which is undoubtedly sufficient for the practical need. This obtains up to an age, hitherto unspecified, beyond which the assumption renders the index insufficiently accurate. It has been found in this investigation that for all teeth combined the assumption entailed with respect to extracted teeth involves an absolute and constant error of 1 per cent up to an age approximating to 25 years. This error gradually increases beyond 25 years until at 62 years it has an absolute value of 24·4 per cent. It thus seems reasonable to conclude that 25 years is the critical age beyond which the error associated with extracted teeth makes the observed DMF values unacceptable.

Vol. 6
1961

This general conclusion also applies to the described individual tooth types, although in the case of molars, extracted teeth involve an error which is relatively insignificant, and in the case of the lower second premolars the absolute error is 4 per cent at 25 years.

It must be stressed that these findings are specific to the community in question and cannot be translated to any other community unless it is currently established that the proportion of carious to non-carious teeth is comparable. By a comparable amendment of the M fraction in the DMF index it should, however, be possible to compare adult prevalence rates between one community and another.

If one accepts the amended DMF values as being a reasonable approximation then several interesting observations are possible. Considering all teeth combined (excluding third molar teeth) there is a gradually diminishing rate of attack as age progresses between 12 and 47 years, with a relatively quiescent period between 32 and 47 years. These values, however, are compounded from prevalence rates of several different tooth types, and more interesting conclusions are possible from an individual study of tooth types. Of particular interest is the existence of relatively quiescent periods between 32 and 47 years, which is most marked in incisor teeth and premolar teeth. For canines there is a relatively short quiescent period from 32 to 37 years, but nevertheless it is present. The situation with respect to molar teeth is not strictly comparable because 90 per cent have already been attacked by the age of 17 years. The fact that a quiescent period commences in the majority of teeth at about the age of 32 years, regardless of individual susceptibilities, appears

to be significant. One possible and obvious explanation is that the more susceptible teeth are attacked rapidly and that attack of the more resistant teeth takes place at a slower rate. This process most likely takes place, but it does not explain the almost critical age at which the rate of attack is so obviously decreased, or the marked increase in rate of attack after this quiescent period. An intriguing possibility is that these remissions and exacerbations in attack are associated with carbohydrate intake. It is known that the intake of carbohydrate in children and adolescents is high, and whereas there is no precise data about carbohydrate ingestion in adults, there is a recognized tendency for adults in mid-life to diet in order to keep their weight down by carbohydrate restriction and to relax this restriction in later life. This explanation must remain speculative in the absence of full facts, but it does offer possibilities of enquiry.

Another interesting fact so often ignored is the remarkable resistance of the lower anterior teeth which remains constant throughout life. From the amended DMF values only 10·6 per cent of the lower incisors are attacked by the age of 47 years, and only 14 per cent by the age of 62 years. Explanations of differing susceptibilities in different tooth types have often been based on mechanical grounds such as morphology and function. The morphological differences between upper and lower incisor teeth are not great and yet the comparable prevalence rates at the ages of 47 and 62 years for upper incisor teeth are 47·3 and 60·8 per cent. With modern diet, the differences in function of upper and lower incisors is not great. The upper premolars have similar morphology and function and have similar prevalence rates. The lower premolars have similar morphology and function but there is a marked difference in prevalence rates between the lower first and second premolars.

Morphology and function do not, therefore, fully explain differences in caries susceptibility. In general, caries susceptibility increases the further the teeth are positioned away from the entrance into the mouth of the lingual saliva, a fact which is constant throughout life. It has been noted by JACKSON (1961) that a breakdown in susceptibility of the lower anterior teeth is associated with a complete breakdown in susceptibility in the rest of the teeth.

It is suggested, therefore, that the high resistance of the lower incisor teeth is associated with some caries inhibiting factor in lingual saliva, which has diminishing influence the further the teeth are situated from its entrance into the mouth. It is also suggested that rampant caries is associated with a diminution or absence of this inhibiting factor.

Acknowledgements — The author wishes to thank Messrs. Thomas Hedley & Co., Ltd. for a research grant facilitating this work. The co-operation of Mr. D. E. TAYLOR, Senior Dental Officer, Leeds Education Authority, and of the respective managements of Messrs. Joseph Watson, Ltd., and Messrs. Montague Burton, Ltd.,

both of Leeds, are deeply appreciated. To Mr. H. G. LUMBY, M.S.I.A., N.D.D., Medical Illustrator, Leeds University and to Miss A. COOPER, the author is indebted for their assistance.

REFERENCES

JACKSON, D. 1950. *Brit. dent. J.* **88**, 207.
JACKSON, D. 1961. In Press.
SLACK, G. L., JACKSON, D., JAMES, P. M. C. and LAWTON, F. E. 1958. *Brit. dent. J.* **104**, 399.
RUSSELL, A. L. and ELVOVE, E. 1951. *Publ. Hlth Rep., Wash.* **66**, 1389.

Vol. 6
1961

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 94–108, 1961. Pergamon Press Ltd.

LONGITUDINAL DENTAL CARIES STUDY IN CHILDREN 9-15 YEARS OF AGE

O. BACKER DIRKS

Organization for Health Research T.N.O., Laboratory of Microbiology, University of Utrecht, Utrecht, the Netherlands

Abstract—A longitudinal caries study was carried out in a group composed of fifty girls and fifty boys. The children, who were born in the same year (1945) and permanent residents of the same town were examined at the age of 9, 11, 12, 13, 14 and 15 years. The yearly examination was done by the same dentists. Special precautions were taken to avoid a shift in the standards of examination. Proximal lesions were recorded from radiograms; pit and fissure lesions and free smooth surface lesions by intra-oral examination.

The number of lesions at the different ages are given separately for each specific surface and in two degrees, i.e. the total number of caries lesions, and the number of lesions which show either a carious involvement of the dentine or a break in the continuity of the enamel surface.

Zusammenfassung—Eine kontinuerliche Kariesuntersuchung an 50 Mädchen und 50 Jungen im Alter von 9–15 Jahren wurde durchgeführt. Die Kinder, welche im selben Jahre (1945) und im selben Ort geboren sind und wohnen, wurden im Alter von 9, 11, 12, 13, 14 und 15 Jahren von den gleichen Zahnärzten untersucht. Eine ständige Kontrolle zur Festhaltung der Untersuchungsmassstäbe wurde durchgeführt. Die Approximalkaries wurde nur im Röntgenbild beurteilt; die Okklusalkaries und die Karies der freien Zahnflächen (bukkal und lingual) in der klinischen Untersuchung.

Die Anzahl der Läsionen wird für jede Altersklasse und für jede Zahnfläche in zwei Stufen gegeben: Gesamtzahl aller Läsionen und separat Dentinläsionen.

INTRODUCTION

STUDIES on the progress of dental caries with age in the same patients (longitudinal caries survey) in which the caries attack is studied for each type of surface separately are relatively scarce. During other studies data were obtained which amounted to such a longitudinal survey. Though they were not intended as such, it seemed worthwile to report these results.

MATERIAL AND METHODS

A group of 50 girls and 50 boys, randomly selected from the school population, were regularly examined at the age of 9, 11, 12, 13, 14 and 15 years. All children were born in 1945 and were permanent residents of the town of Culemborg (Netherlands). As the examinations were carried out in the second half of each year,

the mean age of the group was $9\frac{1}{4}$, $11\frac{1}{4}$ etc. Each examination consisted of two parts, a standardized radiographic examination of proximal surfaces and a standardized intra-oral examination for the pits and fissures and the free smooth surfaces.

The radiographic examination has been performed at the indicated ages, the intra-oral examination only at the ages of 9, 11, 13 and 15 years.

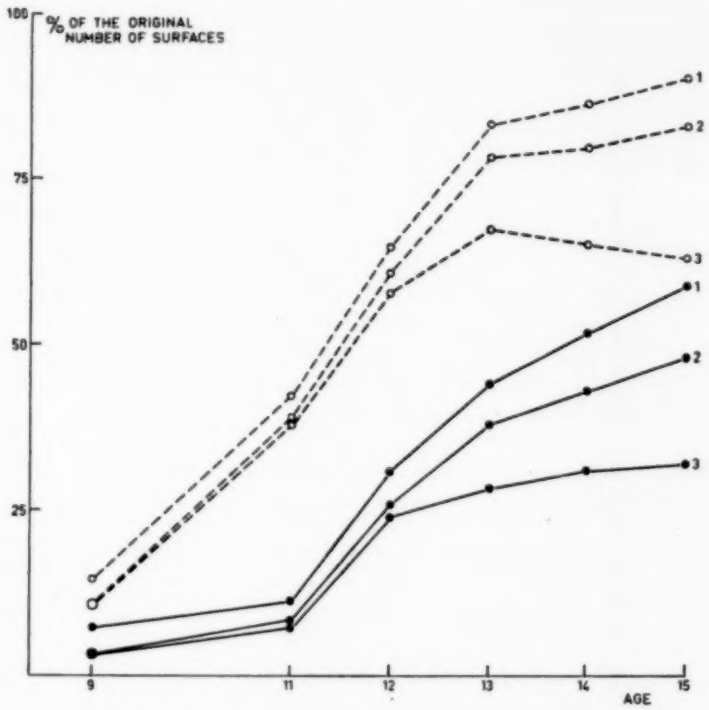


FIG. 1. Percentage of carious lesions of the distal surface of the upper first molar.

In the radiographic survey the lower incisors were not included. The intra-oral examination was limited to the pits and fissures of molars and bicuspids, and with regard to the free smooth surfaces, to the buccal and labial surfaces. The diagnostic methods used in this survey are described elsewhere (BACKER DIRKS, VAN AMERONGEN and WINKLER 1951: BACKER DIRKS and KWANT 1954: BACKER DIRKS, KWANT and KLAASSEN 1957). Special precautions were taken to prevent and check any shift in the diagnostic criteria from year to year (BACKER DIRKS, HOUWINK and KWANT, 1961). This report deals only with lesions in permanent teeth.

The number of lesions are reported in two degrees of severity i.e. all diagnosed lesions (including enamel lesions) and the more severe lesions cavities) which either show carious involvement of the dentin (radiographic examination) or a break

in the continuity of the enamel surface (intra-oral examination). No distinction is made between fillings (past caries lesions) and caries lesions.

In every caries survey the recording of extractions and fillings present special problems. The purpose of the survey will generally decide in which way the extractions are to be counted.

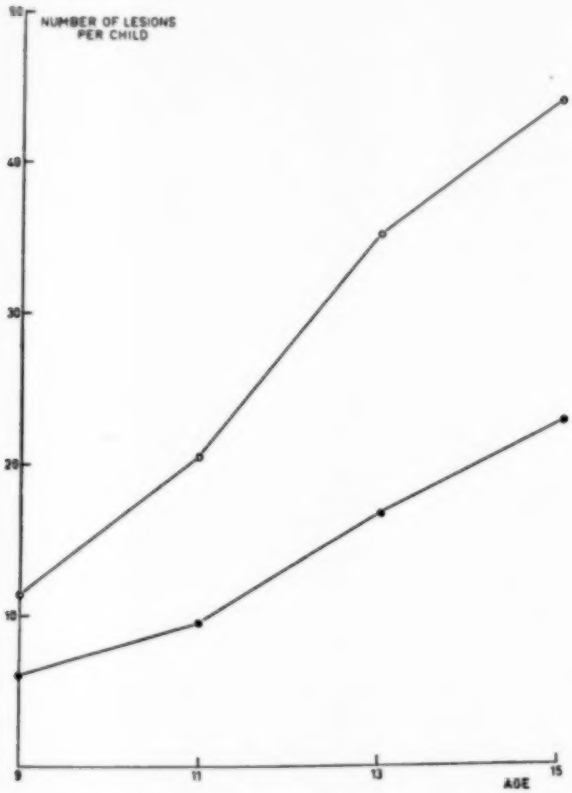


FIG. 2. Number of carious lesions per child (o) and number of cavities or lesions with involvement of the dentine per child (●).

The influence of various ways of scoring extractions can be read from Fig. 1 for the distal surface of the upper first molar. In curve 1 extractions are included scoring all surfaces lost by extraction as carious. In curve 2 extractions are laso included, but scored according to the last observation before the extraction. Curve 3 shows the number of lesions not including extractions. Especially the curves for the surfaces with lesions of the dentin are strongly influenced by the method of scoring extractions.

For a longitudinal survey the second method (curve 2) seems to be the best one. As far as possible this method will be followed here.

The influence on the caries score of the method of scoring the fillings will be discussed in the section on pit and fissure lesions.

Out of the vast amount of data only a selection can be reported. For instance the data for boys and girls will only occasionally be given separately.

RESULTS

The caries score in number of lesions per child at the age of 9, 11, 13 and 15 years, is given in Fig. 2. These combined data give a poor impression of the true progress of caries, as the contribution of proximal caries and fissure caries to the total score is quite different at various ages. Moreover the scoring method overemphasizes the contribution of pit and fissure caries as the two fissures of the upper molars and the occlusal fissure and the buccal pit of the lower, molars are scored separately. The separate data for the diffrent type of lesions will be more informative.

Vol. 6
1961

(a) *Pit and fissure lesions*

The number of pit and fissure lesions at the different ages is shown in Table 1 and Fig. 3. All curves show a rather steep increase in the number of caries lesions over the whole period from 9 through 15 years.

The boys show lower caries numbers than the girls. Between the age of 13 and 15 the boys show about the same number of cavities as the girls one year younger. The difference in the total number of lesions is (including "incipient caries") less pronounced.

At the age of 9 the number of lesions is already very high in comparison with the number of pits and fissures at risk. Consequently even before this age the curves must have been steep.

For a more detailed understanding of these data, Table 2 gives the number of cavities per 100 children for each specific class of fissures.

At the age of 9 only an insignificant fraction of the caries score is contributed by other teeth than the first molars. The occlusal fissure of the lower molar leads, followed by the mesial and distal fissure of the upper molar.

Figure 4 pictures the increase in the number of cavities during each two-year-period for first and second molars and bicuspids separately (the increment for the period 5–7 and 7–9 years is assessed from the caries increments of similar groups of children).

Up to the age of eleven almost the total caries increment is due to lesions in the first molar. The increased slope of the caries curve after the of 11 is due to lesions in the second molar and to a lesser extent in the bicuspids.

The second molar erupts 6 years after the first molar; at the age of 15 the second molar attains the same post-eruptive age as the first molar at 9 years. The number of cavities of the first molar at 9 years is about equal to that of the second molar

TABLE 1. PIT AND FISSURE CARIES EXPERIENCE OF MOLARS AND BICUSPIDS YEAR OF BIRTH 1945

Age (years)	Sex	No. of pits and fissures*	No. of carious lesions†	No. of cavities†
9	♀♀	9·5	6·5	5·3
	♂♂	9·8	6·3	4·8
11	♀♀	17·1	9·5	7·2
	♂♂	15·9	9·0	6·5
13	♀♀	22·4	14·8	11·8
	♂♂	22·0	13·5	9·9
15	♀♀	23·0	17·1	14·1
	♂♂	23·2	16·5	12·7

* Including pits and fissures lost by extraction.
† Extractions are counted as the last diagnosis before the extraction.

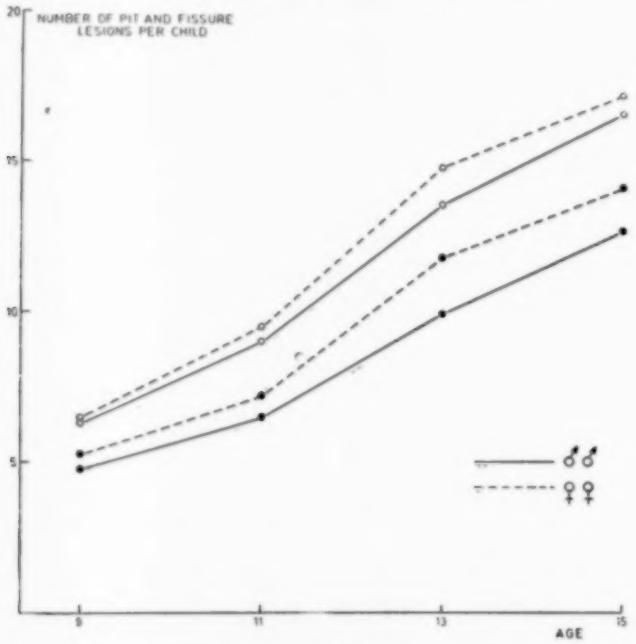


FIG. 3. Number of pit and fissure lesions per child. o, Total number of lesions; ●, Number of cavities. Boys: full drawn lines; Girls: dotted lines.

at 15 years (Table 2). This is especially striking for lower molars. For upper molars there is a difference in caries number of the disto-occlusal fissure. This difference might be explained by the anatomical form of the disto-occlusal fissure of the upper second molar which is very shallow and occasionally absent. Apart from this difference, first and second molars seem to have the same caries susceptibility.

TABLE 2. NUMBER OF PIT AND FISSURE CAVITIES (TOTAL OF LEFT AND RIGHT MOUTH HALF) PER 100 CHILDREN

Age (years)	Upper						Lower					
	P_1	P_2	M_1		M_2		P_1	P_2	M_1		M_2	
	o	o	mo	do	mo	do	o	o	o	b	o	b
9	5	2	146	121	0	0	0	3	170	54	0	0
11	13	15	173	160	5	1	2	9	182	78	25	9
13	46	45	192	186	92	38	17	34	191	83	71	31
15	64	70	198	189	143	89	23	57	192	97	162	45

o = occlusal fissure
mo = mesio-occlusal fissure
b = buccal pit
do = disto-occlusal fissure
For extracted molars the caries diagnosis preceding the extraction is used.

Vol. 6
1961

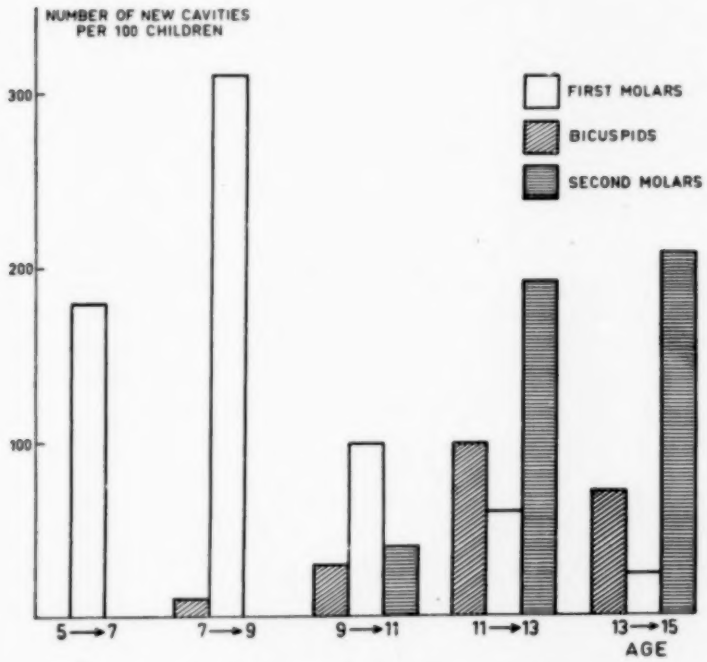


FIG. 4. Increment of pit and fissure cavities.

The bicuspids, erupting somewhat earlier than the second molars, show a far lower attack rate.

The caries score of the occlusal fissure of the bicuspids is strongly affected by the number of proximo-occlusal fillings inserted for proximal lesions. At the age of 15 years, 19 per cent of the upper bicuspids which are scored for occlusal caries

(cavity or filling) show a proximo-occlusal filling. For the lower first and second bicuspid this percentage is resp. 13 and 28 per cent. These percentages are a measure for the inaccuracy of the occlusal score of these bicuspids. At the age of 13 these percentages are smaller, i.e. 10 per cent for the upper bicuspids and resp. 5 and 16 per cent for the first and second bicuspid.

If we make the supposition that the percentage of occlusal cavities was the same in bicuspids with proximal fillings and without proximal fillings, the caries score of the bicuspids would decrease. Under this supposition the number of cavities as given in Table 2, would become at 15 years for the lower first and second bicuspids 20 and 46 and for the upper bicuspids 56 and 62.

For the molars there is no such problem as all occlusal fissures of the first molar were already carious at the age of 9 years.

The difference in caries score between girls and boys seems not only to be determined by an earlier eruption but also by another factor. If the time of eruption was the only factor, the first molars of girls and boys would have attained the same caries level at 15 years. Moreover, although the premolars erupted at about the same time in the girls and the boys, the premolars of the girls show a 20 per cent higher attack rate than the boys.

(b) *Proximal lesions*

The curves of Fig. 5 show the number of proximal lesions per child from 9 through 15 years for girls and boys separately. The difference in number of lesions between girls and boys is slight in contrast to the caries attack in pits and fissures. The shape of the curves is also different from those in Fig. 3. In Fig. 5 the curve for lesions affecting the dentin lags behind the curve for all lesions (including enamel lesions) with a time interval of about 3 to 4 years. This means that caries develops slowly, as it takes—in the mean—3–4 years for an incipient lesion to develop to a lesion affecting the dentin. The rate of progression of initial pit and fissure caries is much larger and the period for development to a cavity is less than one year at least up to the age of 13 years.

From 9 through 13 years of age the increase in caries numbers is very high, after 13 years the increase is smaller. At the age of 15 years, 50 per cent of the proximal surfaces are affected. The increment in the number of surfaces with involvement of the dentin from 9 to 12 years is smaller than that from 12 to 15 years.

The distribution of the lesions over the various surfaces for the nine-years-old group is shown in Fig. 6. The caries distribution at the age of 15 is shown in Fig. 7, whereas Fig. 8 pictures for the same age the distribution of the lesions in which the dentin is also involved. In these there Figs. 6, 7 and 8, the *mean* of the right and left mouthhalves are given. The numbers on the top of the bars for the first molars indicate the number of carious surfaces, that were lost by extraction.

Vol. 6
1961

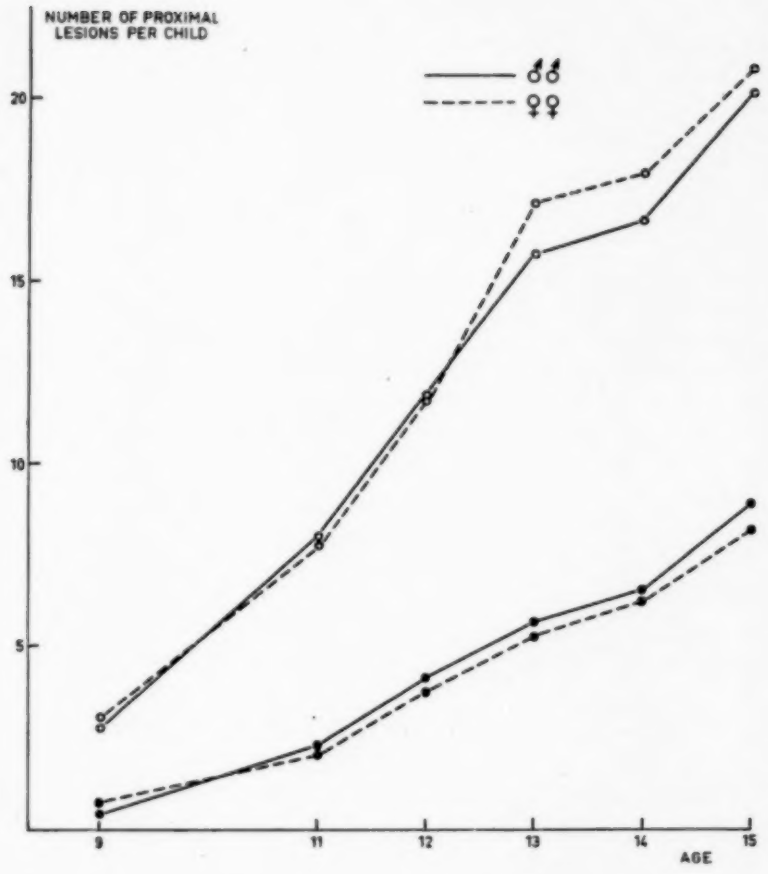


FIG. 5. Number of proximal lesions per child. ●, Total number of lesions; o, Number of lesions with involvement of the dentine. Boys: full drawn lines; Girls: dotted lines.

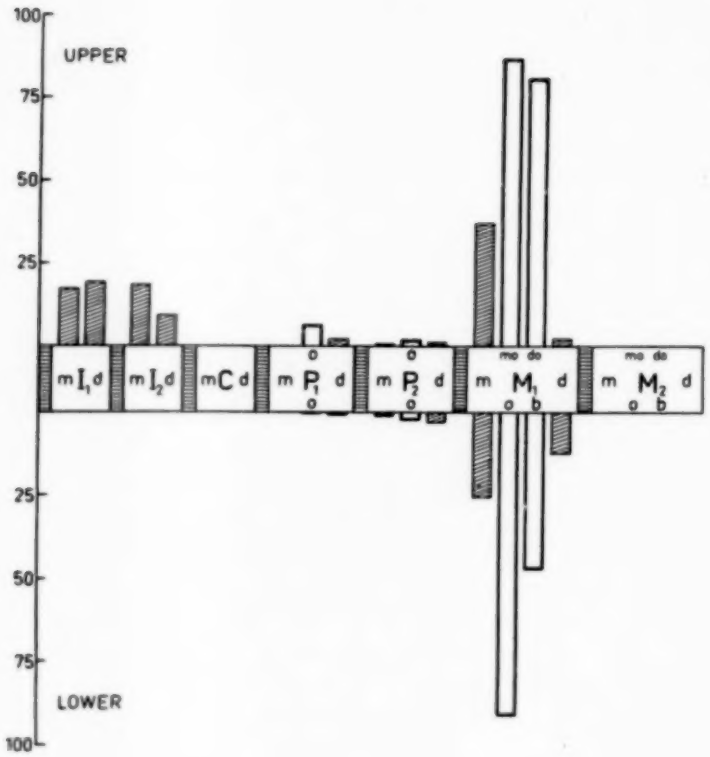


FIG. 6. Number of lesions per 100 children at 9 years of age. Mean of left and right halves of the mouth.

I_1	= first incisor	d	= distal surface
C	= cuspid	o	= occlusal fissure
P_1	= first bicuspid	mo	= mesio-occlusal fissure
M_1	= first molar	do	= disto-occlusal fissure
m	= mesial surface	b	= buccal pit.

The top of the molar bars indicate the number of lesions that were lost as a consequence of extraction.

Vol. 6
1961

Vol. 6
1961

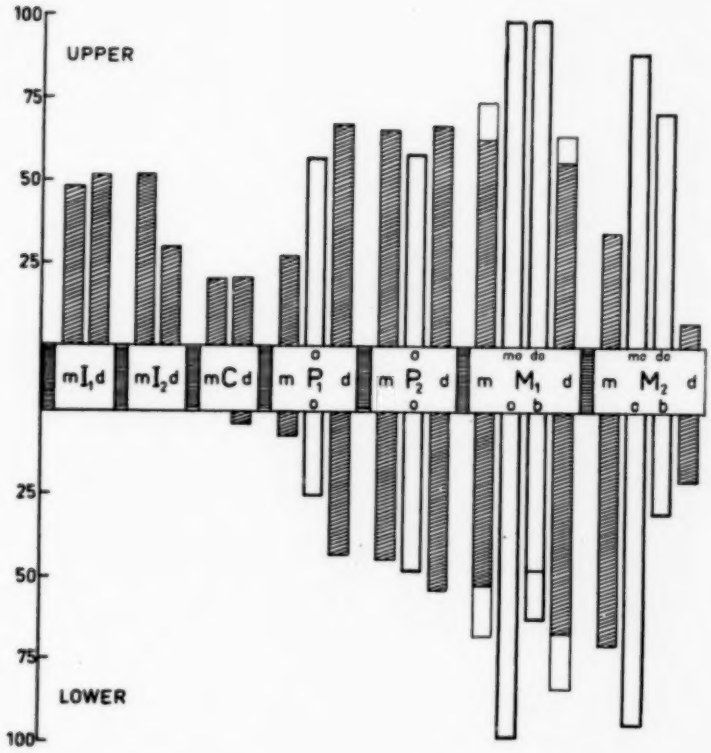


FIG. 7. Number of lesions per 100 children at 15 years of age. Mean of left and right halves of the mouth. For legend see Fig. 6.

Table 3 shows the attack of separate surfaces—in two degrees—as the *sum* of the right and left mouthhalves at the various ages. The difference in number between the two classes of lesions is the number of lesions that—as far as visible on the X-ray—are confined to the enamel. Numerically they are a very important fraction of the total number of lesions; at 9 years 80 per cent of the lesions are enamel lesions and at 15 years 58 per cent.

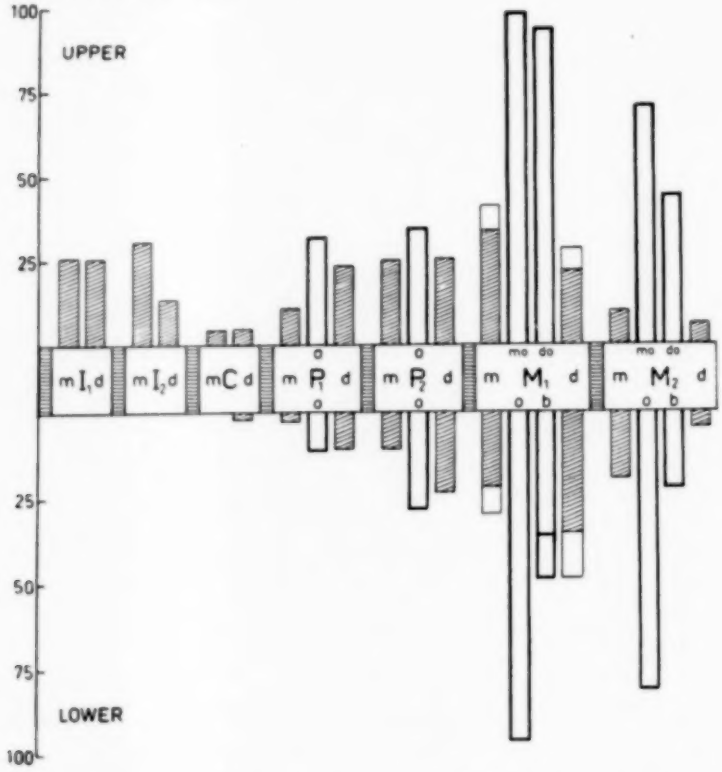


FIG. 8. Number of cavities (for pits and fissures) and of carious lesions with involvement of the dentine (proximal surfaces) at 15 years of age. Mean of left and right halves of the mouth. For legend see Fig. 6.

The Figs. 9 and 10 show the number of lesions for each proximal surface of the maxillary teeth. Three types of curves can be distinguished. The first type are the curves for the surfaces which had already an important number of lesions at nine years. These curves show a moderate slope up to the age of 13, after this age the curve has an insignificant slope. The mesial and distal surfaces of the first incisor, the mesial surface of the second incisor and the mesial surface of the first molar belong to this type. The only difference is the far higher level of the molar curve.

Vol. 6
1961

TABLE 3. NUMBER OF PROXIMAL LESIONS (TOTAL FOR LEFT AND RIGHT HALVES OF THE MOUTH) PER 100 CHILDREN

Caries grade	Age (years)	Upper																Lower										Total number of lesions per child
		I_1		I_2		C		P_1		P_2		M_1		M_2		C	P_1		P_2		M_1		M_2					
		m	d	m	d	m	d	m	d	m	d	m	d	m	d	d	m	d	m	d	m	d	m	d				
All lesions	9	34	39	37	19	1	0	0	4	1	3	75	5	0	0	0	0	0	1	6	52	24	0	0				3·01
	11	59	71	71	29	3	6	11	37	42	55	102	21	5	0	1	2	16	21	41	85	79	35	3				7·95
	12	78	87	89	35	12	14	15	71	72	83	122	54	18	3	0	2	37	32	53	89	122	83	11				11·82
	13	89	99	99	47	25	26	37	95	93	116	138	95	40	8	7	13	61	57	85	114	154	116	33				16·47
	14	87	98	93	50	29	33	41	107	110	117	138	104	52	6	7	14	69	67	90	114	157	126	24				17·33
	15	96	104	104	60	41	41	55	135	132	134	148	128	70	15	7	15	88	90	108	135	167	140	41				20·54
Lesions with involvement of the dentine	9	7	7	7	4	0	0	0	0	0	0	10	1	0	0	0	0	0	0	1	17	5	0	0				0·59
	11	30	30	29	9	0	1	1	2	3	6	38	6	0	0	0	0	1	1	7	30	20	1	0				2·15
	12	40	44	41	13	1	2	5	12	17	22	55	18	1	1	0	1	5	4	17	40	48	5	3				3·95
	13	42	48	50	16	2	6	7	27	25	37	65	34	7	2	1	5	8	9	23	51	71	10	4				5·50
	14	45	51	52	20	6	7	11	32	34	39	72	48	13	3	1	6	13	13	29	50	80	13	4				6·42
	15	52	52	62	27	9	12	22	47	50	52	83	57	20	13	4	6	21	22	47	60	96	40	4				8·58

m=mesial surface
d=distal surface
For extracted teeth the caries diagnosis preceding the extraction is used.

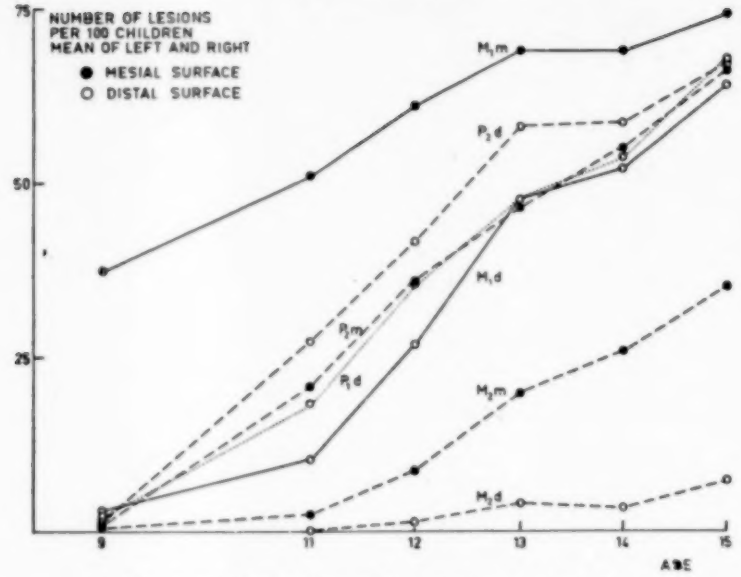


FIG. 9. Proximal lesions of the upper posterior teeth.
P_1=first bicuspid
M_1=first molar
m=mesial surface
d=distal surface.

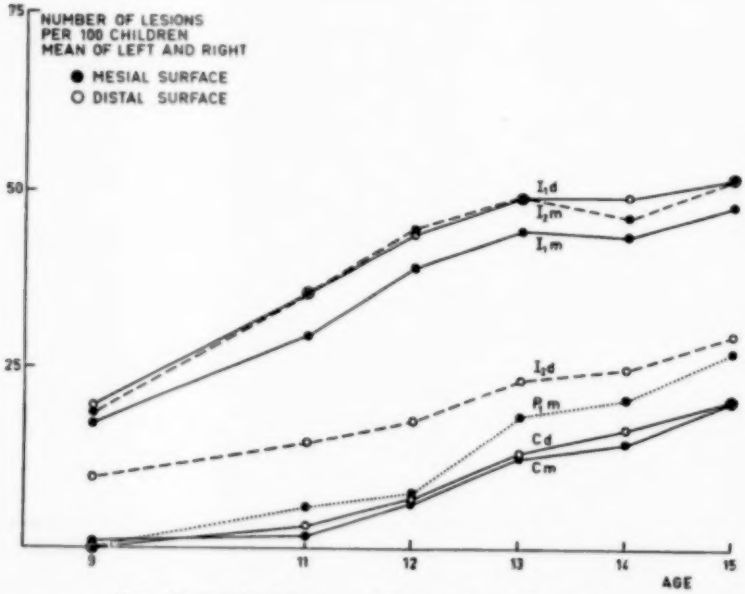


FIG. 10. Proximal lesions of the upper anterior teeth.
I_1 =first incisor
C =cuspid
P_1=first bicuspid
m=mesial surface
d =distal surface.

The premolar surfaces, except the distal surface of the second premolar, and the distal surface of the first molar show an other type of curve. Starting at nine years at zero, a very steep rise follows, which brings the curves to about the same height as the mesial surface of the first molar. The curves of the proximal surfaces of the cuspid and the adjacent surfaces of first premolar and second incisor show the smallest slope. The mesial surface of the second molar seems to have the same type of curve as the mesial surface of the first molar.

In general the caries numbers of two adjacent surfaces show more similarity than the two surfaces of the same tooth. There is therefore perhaps more reason to describe the caries pattern with the interproximal space as the unit, than with the tooth as the unit. The dissimilarity of adjacent surfaces in the younger age groups tends to become smaller each subsequent year. This is especially true in those adjacent surfaces which show an important difference in time of eruption (e.g. M_1m—P_2d).

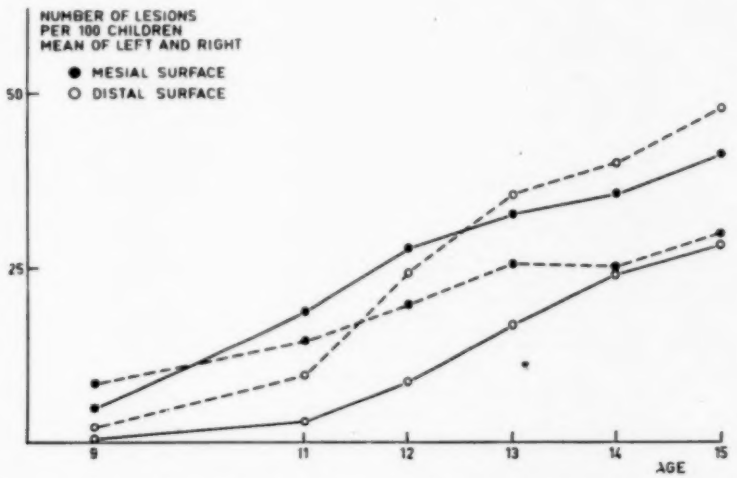


FIG. 11. Proximal lesions with involvement of the dentine. First molar. ●, Mesial surface; o, Distal surface. Upper molar: full drawn lines; Lower molar: dotted lines.

Figure 11 brings together the four surfaces of the upper and lower first molar. The number of surfaces with lesions of the dentin are shown. Each of these four curves shows a different shape. At nine years both mesial surfaces show a higher caries score than the distal surfaces. However, at the age of 15 the distal surface of the lower molar has a much higher caries number than its mesial surface. The distance between mesial and distal surface of the upper molar curves is fairly constant.

At the age of $10\frac{1}{2}$ years 50 per cent of the upper second premolars were erupted, for the lower premolars this age was $11\frac{1}{4}$ years. The upper and lower second molars

were for 50 per cent erupted at resp. 12 and 11 years. The presence of the deciduous molars explains the differences at 9 years; the eruption sequence of the permanent premolars and molars explains, at least partly, the different caries rates after 9 years.

(c) *Buccal and labial smooth surface lesions*

The lesions of the buccal or labial surfaces are diagnosed only in the course of the examinations at 13 and 15 years.

Those surfaces were diagnosed as carious, that after cleaning and drying with compressed air during two to three seconds, either showed a white, chalky, non glossy enamel area, or a break in the enamel, perceptible by the sharp explorer.

TABLE 4. NUMBER OF BUCCAL AND LABIAL LESIONS PER CHILD

	Age (years)	
	13	15
No. lesions	5·3	8·0
No. cavities	0·9	1·7

Table 4 gives the number of buccal lesions in two classes.

Except for the first molars all teeth showed an increment in carious lesions. However, it is not possible upon the basis of these two examinations to draw any further conclusions.

REFERENCES

BACKER DIRKS, O., HOUWINK, B. and KWANT, G. W. 1961. The results of 6½ years of artificial fluoridation of drinking water in the Netherlands. The Tiel–Culemborg experiment. *Arch. oral Biol.* **5**, 284–300.

BACKER DIRKS, O. and KWANT, G. W. 1954. A reproducible method for caries evaluation—IV. *Tijdschr. Tandheelk.* **61**, 891–904.

BACKER DIRKS, O., KWANT, G. W. and KLAASSEN, C. B. 1957. A reproducible method for caries evaluation—V. *Tijdschr. Tandheelk.* **64**, 77–85.

BACKER DIRKS, O., VAN AMERONGEN, J. and WINKLER, K. C. 1951. A reproducible method for caries evaluation. *J. dent. Res.* **30**, 346–359.

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 109–118, 1961. Pergamon Press Ltd.

CHANGES IN THE DEPTH OF ARTIFICIAL MARKS ON TOOTH SURFACES AFTER CERTAIN TIME INTERVALS*

P. SCHLATTER, T. M. MARTHALER and H. R. MÜHLEMANN
Dental Institute, University of Zurich, Switzerland

Vol. 6
1961

Abstract—In order to study both morphological characteristics and depth changes of marks engraved *in vivo* on tooth surfaces, two replicas were made. One replica was used for surface microscopy, the other was imbedded in wax and cut serially on a microtome at a right angle to the mark. In the sections obtained, the depth of the mark was measured under the microscope.

The depth changes were studied in thirty-four students. Some marks were found to be deeper after 1 week. After 6 weeks, the average depth of the marks was smaller than at the beginning of the experiment. In the material studied, no relation existed between the depth changes on one hand and oral hygiene, the DMFS and the diet on the other. The correlation between depth data in sections and breadth determinations by surface microscopy was poor.

Zusammenfassung—Um Veränderungen der Tiefe von artifiziell an der Schmelzoberfläche *in vivo* erzeugten Rillen zu bestimmen, wurden zwei Replicapräparate hergestellt. Ein Präparat wird für die oberflächenmikroskopische Untersuchung benutzt, das andere wird in Wachs eingebettet und mit dem Mikrotom im rechten Winkel zu der Rille in Serien geschnitten. In den so erhaltenen Schnitten wird die Tiefe der Rillen unter dem Mikroskop gemessen.

Die tiefenveränderungen an den standardisierten künstlich erzeugten Rillen wurden bei 40 Studenten untersucht. Es wurde festgestellt, daß alle Rillen nach einer Woche tiefer geworden waren. Nach 6 Wochen war die durchschnittliche Tiefe der Rillen geringer als zu Beginn. Die Einebnung der Rillen erfolgte unterschiedlich schnell. Die Beziehungen zwischen den an den Schnitten und an den oberflächenhistologischen Bildern erzielten Ergebnisse waren nicht eindeutig. Es konnte keine Abhängigkeit zwischen den Veränderungen der Rillentiefe, dem DMF-Flächenindex und der Nahrung bei den untersuchten Studenten gefunden werden. Dies ist möglicherweise auf die Unterschiede bei der Zahnpflege zurückzuführen. Die Veränderungen in der Tiefe der künstlichen Rillen wird durch nicht entfernbare Ablagerungen und durch die Abrasion der Schmelzoberfläche verursacht.

INTRODUCTION

THE REPLICA technique ("adhesion relief method") has been introduced by WOLF (1939) for the assessment of the *in vivo* behaviour of the tooth surface. The description of the structured details as seen on replicas was extended by observations

* Supported in part by the Swiss Odontological Society and the Colgate-Palmolive Company

of SCOTT and WYKOFF (1946). PEDERSON and SCOTT (1951) found differences in enamel surface appearance between American natives and Alaskan Eskimos.

WOLF and NEUWIRTH (1941) showed the disappearance of scratches, produced *in vivo* on the tooth surface, within 1 or 2 weeks. MANNERBERG (1960), who surveyed the enamel replica literature thoroughly, published a monograph on the appearance of tooth surfaces in various age groups, in long-term studies after tooth brushing, in cases of erosion and after exposure to citrus fruit juice. Tooth surface morphology varied more with toothbrushing habits than with age. Old scratches disappeared simultaneously with the appearance of new ones. Such changes may be due to a continuous diffuse abrasion of the tooth surface and/or to a reparative precipitation of deposits into artificial marks and tooth brush scratches.

It is conceivable that the rate of disappearance of artificial marks might be used for the study of the physico-chemical equilibrium between tooth surface and saliva, since it seems likely that the deposits within the scratches and marks consist of inorganic material (MANNERBERG (1960): "probably calcium phosphate"; KNAPPWOST (1956): "hydroxyapatite").

One of the shortcomings of the commonly used replica method is the unreliable determination of the depth of marks engraved on the tooth surface. MANNERBERG (1960), who used a calibrated engraving instrument for the production of marks, determined their depth by trigonometrical calculations from breadth measurements under the miscroscope.

It was the purpose of the present study to compare findings obtained from the surface inspection of replicas with measurements of the actual depth of the engraved mark.

MATERIAL AND METHOD

Vertical marks were engraved on the labial surface of both upper incisors by means of a diamond point having an angle of 100° and using a standardized force. Afterwards, replicas were taken in duplicate on the rubberdam isolated incisors. Details have been described elsewhere (SCHLATTER, 1962).

One of the replicas was used for surface microscopy (oblique illumination). The other replica was sandwiched between two wax plates and sectioned on the microtome at 25 μ thickness and at a right angle to the mark, in the area of the first millimeter of its incisal end. The depth of the mark was then measured under the microscope in twenty sections mounted on a slide and previously treated with xylene.

The error of the method was determined by measuring the depth of the marks in twenty replicas made in duplicate and sectioned independently. Variance and standard deviation of the method were 0·0955 and 0·309 (Table 1). As is to be shown under the results, this method error is considerably smaller than the individual variance of the scratch depth. Thus the method error contributes only little

to the experimental error, and the method may therefore be judged as being sufficiently precise.

Replicas were taken immediately after the mark was made, 1 and 6 weeks later.

TABLE 1. ANALYSIS OF VARIANCE OF THE 204 DEPTH DETERMINATIONS IN UPPER CENTRAL INCISORS OF THIRTY-FOUR STUDENTS

Source of variation	Degrees of freedom	Mean square
Subjects	33	3·37*
Times	2	7·02*
first vs. second	1	0·10
first + second vs. third	1	13·93*
Subject × times	66	1·34
Total subject + times	101	—
Left vs. right incisors	1	1·48
Interaction	101	1·08
Total measurements	203	—
Error of measurement†	20	0·0955

* Significantly greater ($P<0.01$) than interaction subject × times.
† See "Material and Method".

RESULTS AND DISCUSSION

In thirty-four students, the average depth of the freshly engraved mark was $5{\cdot}07 \pm 1{\cdot}039\ \mu$ (S. D.) for the upper right and $5{\cdot}34 \pm 0{\cdot}842\ \mu$ for the upper left incisor, the difference between right and left being insignificant (Table 2). After 1 week, the average depth in the right incisor had increased by $0{\cdot}16\ \mu$ and decreased

TABLE 2. DEPTH OF MARKS ENGRAVED ON THE LABIAL OF UPPER INCISORS. *In vivo* MEASUREMENTS IMMEDIATELY, 1 AND 6 WEEKS AFTER ENGRAVING

Depth	No.	Incisor	
		Right	Left
Initial	34	5·07±1·039	5·34±0·842
1 week	34	5·23±1·490	5·27±1·670
6 weeks	34	4·57±1·450	4·77±1·346

in the left incisor by $0{\cdot}07\ \mu$, these differences again not being significant. After 6 weeks, the depth of the mark was measured to be, in the right incisor, $4{\cdot}57 \pm 1{\cdot}450\ \mu$ and, in the left incisor, $4{\cdot}77 \pm 1{\cdot}346\ \mu$. In an analysis of variance combining the findings in both incisors, the decrease in depth of the mark was found to be significant (Table 2). In some cases, a striking increase in depth of the mark was observed after 1 week (Fig. 2).

Vol. 6
1961

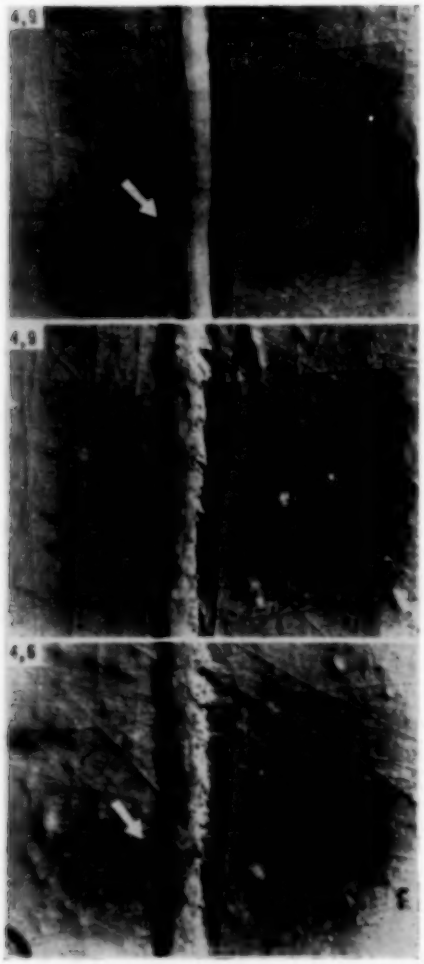


FIG. 1. Artificial mark on labial of upper left incisor. *Upper*: Fresh mark. Clean bottom with lines parallel to the mark. Several toothbrush scratches on otherwise intact crown surface. Indentations due to splitting off of surface enamel with appearance of prism ends. Depth of scratch 4·9 μ. S 132–36, orig. magn. $\times$64. *Middle*: Same mark after 1 week. Rather smooth appearance of bottom, disappearance of parallel lines. Increase in size of indentations. Depth 4·9 μ, S 132–37. *Lower*: Same mark after 6 weeks. Smooth edge of mark with incorporated indentations. Depth 4·6 μ. Pattern of toothbrush scratches identical in all three replicas.

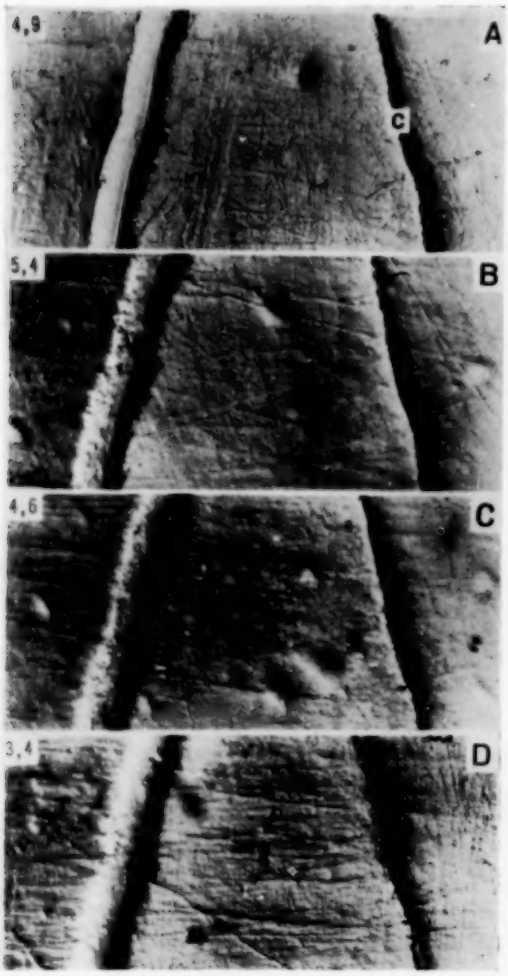


FIG. 2. Artificial mark on labial of upper left incisor. C=crack. A: Fresh mark. Indentations at the edge of the mark. B: Same mark after 1 week. Smooth appearance of bottom and margin of edge. Depth 5·4 μ. C: Same mark after 6 weeks. Indentations incorporated into mark. Depth 4·6 μ. D: Mark after 12 weeks. Depth 3·4 μ. Pattern of toothbrush scratches constantly modified during experimental period. S 132–39/42, orig. magn. ×64.

The morphological changes occurring in the marks are represented in Figs. 1–5. The fresh marks always showed a clean bottom with several lines parallel to the main scratch and due to imperfections in the diamond point (Fig. 4). The

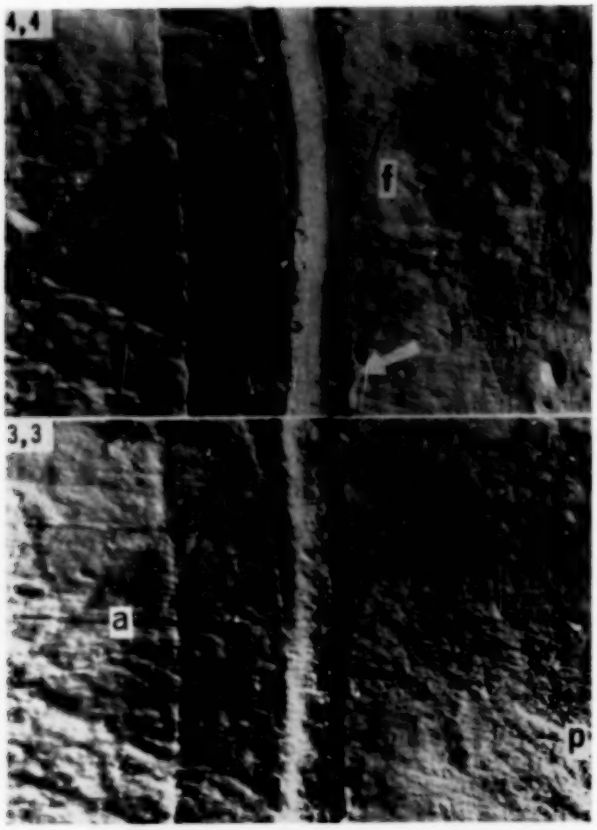


FIG. 3. Artificial mark on labial of upper right incisor of fresh crack. *Upper*: Fresh mark. Splitting off of enamel at the edge of the mark. Prism ends visible in indentations. Depth 4·4 μ. *Lower*: Mark after 6 weeks showing plaque-like honeycomb pattern on the bottom of mark. Incorporation of edge indentations. p = plaque a = artefact. Depth 3·3 μ. No toothbrush scratches. S 130–18/19, orig. magn. ×64.

margins of the mark were well outlined but in many instances notched. These identations were produced by the splitting off of surface enamel during the engraving of the mark. These fracture regions often revealed the morphological pattern of prism ends (Figs. 1, 3).

After 1 week, the artificial marks generally appeared to be of smoother outline, the marginal identations being gradually incorporated into the main mark (Figs. 1, 2). The parallel lines on the bottom of the mark had disappeared in all instances

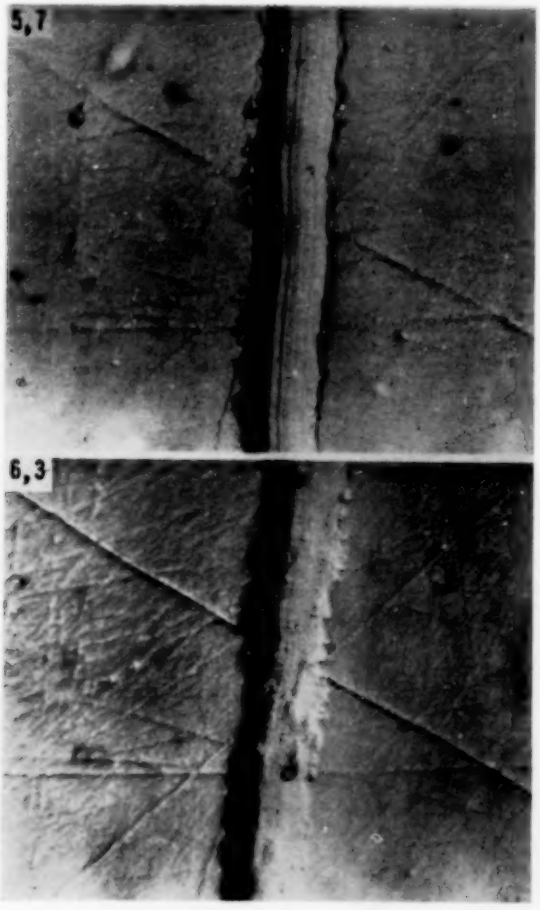


FIG. 4. Artificial mark on labial of upper left incisor. *Upper*: Fresh mark with parallel lines at the bottom. Splitting-off of enamel at the edge of the mark. Depth 5·7 μ. *Lower*: Mark after 6 weeks. Disappearance of parallel lines. Smooth appearance of bottom of mark. Unchanged pattern of toothbrush scratches. S 129–31/32, orig. magn. $\times$ 102.

(Figs. 1, 2, 5). The bottom showed, in some cases, a surface texture similar to that of the adjoining intact enamel (Figs. 1, 2), in other cases an irregular, plaque-like roughness (Fig. 5).

Vol. 6
1961

FIG. 5. Artificial mark on labial of upper right incisor. *Upper*: Fresh mark. Depth 4·5 μ. *Middle*: Mark after 1 week. Disappearance of parallel lines on bottom of scratch. Depth 5·6 μ. *Lower*: Mark after 6 weeks. Plaque-like deposits on bottom of mark. Depth 3·0 μ. Unchanged pattern of toothbrush scratches. S 131–15/17, orig. magn. $\times$64.

Vol.
196

After 6 weeks, the surface structure within the marks was similar to the one observed after 1 week. In general, the borders of the mark had become still smoother. On the bottom of a few marks, a honeycomb pattern could be observed (Fig. 3).

The pattern of toothbrush scratches in many instances remained identical during the experimental period (Figs. 1, 4, 5). In three cases, a decrease in the depth of the marks therefore was hardly due to abrasion of the tooth surface, but rather to deposits of organic (plaque-like deposits? Fig. 5) or of inorganic (?) nature (Figs. 2, 3). In other cases, the depth of the mark remained unchanged or even increased during the 6 week period (Fig. 4). It is interesting to note that in such marks, a similar structure was found at the bottom of the mark as was observed in the marks having decreased in depth (Fig. 4 to be compared to Fig. 1).

Vol. 6
1961

No correlation was found between the depth changes of the marks and good or improper oral hygiene, caries resistance or activity, and diets with or without frequent consumption of fruit juices.

Measurements of the breadth of the marks made with a standardized instrument have been used by MANNERBERG (1960) in order to calculate the depth of the mark. Similar measurements of breadth were made on fresh marks in thirty replicas of

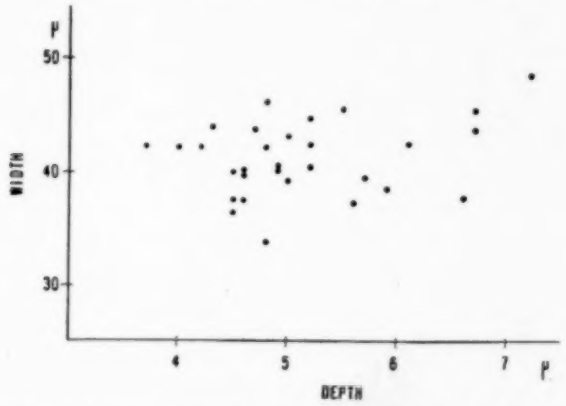


FIG. 6. Correlation between width (breadth) and factual depth of marks engraved on tooth surfaces with a diamond point. Measurements on replicas.

the present material. The findings were poorly related to the actual depth determinations of the mark (Fig. 6). This might be due to an inadequate angle symmetry of the engraving diamond point used in the present investigation or to optical errors of observation when determining the breadth of the marks. The latter assumption seems to be the more probable one.

The study has shown beyond any doubt that the decrease in the depth of marks is of different rate in different individuals, and that it is produced, during a 6 week

experimental period, rather by deposits in the marks than by diffuse surface abrasion by dentifrice abrasives. However, the nature of the deposits is still a matter of speculation.

REFERENCES

KNAPPWOST, A. and EFFINGER, A. 1956. Experimentelle Untersuchungen zur Resistenztheorie der Karies. *Dtsch. zahnärztl. Z.* **11**, 669.

MANNERBERG, F. 1960. Appearance of the tooth surface. *Odont. Revy* **11**, Suppl. 6.

PEDERSON, P. D. and SCOTT, D. B. 1951. Replica studies of the surfaces of teeth from Alaskan Eskimos, West-Greenland natives and American whites. *Acta odont. scand.* **9**, 262.

SCHLATTER, P. 1961. Untersuchungen der sog. Remineralisation mechanischer Schmelzläsionen. Thesis, Zürich. In press.

SCOTT, D. B. and WYKOFF, R. W. G. 1946. Shadowed replicas of tooth surfaces. *Publ. Hlth Rep., Wash.* **61**, 697.

WOLF, J. 1939. Ueber die Herstellung mikroskopischer Präparate der Oberflächen verschiedener Objekte mit Hilfe der Adhaesionsmethode. *Z. Wiss. Mikr.* Heft **2**.

WOLF, J. and NEUWIRTH, F. 1941. Reparative Vorgänge an der Oberfläche natürlicher und künstlich hervorgerufener Schmelzdefekte. *Dtsch. Zahn-, Mund- u. Kieferheilk.* **8**, 222, 349.

Vol.
196

Arch. Oral. Biol., Special Supplement, Vol. 6, pp. 119–124, 1961. Pergamon Press Ltd.

THE EFFECT OF VARIOUS ANIONS ON THE SOLUBILITY OF ENAMEL AND DENTINE IN ACID

S. A. LEACH

Turner Dental School, University of Manchester, England

Abstract—The amount of mineral that is dissolved at equilibrium by reaction of an acid with powdered enamel and dentine at an acid pH should, unless there is some specific interaction between the anion of the acid and the mineral matter, be accounted for in terms of the chemical properties of the solution and of the anion. The following acids were tested for specific interaction effects: alanine, aspartic, citric, glutamic, lactic, malic, malonic, succinic and valine.

The decalcifying solutions contained 0·2 M acetic acid–sodium acetate buffer, 1 M sodium chloride and each of the above acids in turn whose total acid and various anion concentrations were 0·1 M in each case.

With the single exception of citric acid, whose specific interaction with this mineral is already well established, the final pH and the amount of decalcification correlated with the buffering capacity of each solution.

Zusammenfassung—Die Menge an Salzen, die im Gleichgewichtszustand von einer Säure aus pulverisiertem Schmelz und Dentin bei saurem pH herausgelöst wird, müßte auf Grund der chemischen Eigenschaften der Lösung und des Anions berechnet werden können, wenn nicht irgendeine spezifische Reaktion zwischen dem Säureanion und den Zahnmineralien auftritt. Folgende Säuren wurden auf eine solche spezifische Reaktion hin getestet: Alanin, Asparaginsäure, Zitronensäure, Glutaminsäure, Milchsäure, Maleinsäure, Mallonsäure, Bernsteinsäure und Valin.

Die Entkalkungslösung enthielt 0,2 M. Essigsäure–Natriumacetat–Puffer, 1 M. NaCl und abwechselnd jede der oben genannten Säuren mit einer gesamten Säure- und Anionenkonzentration von 0,1 M.

Mit der alleinigen Ausnahme von Zitronensäure, deren spezifisches Verhalten gegenüber den Zahnmineralien bereits hinreichend nachgewiesen wurde, entsprach der pH-Endwert und die Stärke der Entkalkung der Pufferkapazität jeder Lösung.

INTRODUCTION

IN COMPARATIVE studies of the decalcification of enamel and dentine by several organic acids it is generally found that some acids are relatively better than others in their ability to dissolve the mineral matter from these tissues. The relative abilities of these acids to decalcify is not a constant, however, and varies under different experimental conditions. This has produced what might appear to be discrepancies between the results obtained from different laboratories, and has led some workers to conclude that some of these acids are able to influence the decalcification of the mineral matter by some mechanism other than that provided by the acidolytic action of the various decalcifying acid buffer solutions (MCCLURE and RUZICKA,

1946; BUONOCORE and SOMMER, 1954, 1957; LITTLE, BUONOCORE and SOMMER, 1954; KOULOURIDES and BUONOCORE, 1958; BUONOCORE, 1958; WYNN, HALDI and LAW, 1960). The object of this work is to present the results of some studies on the solubility of powdered enamel and dentine in various acid solutions made up of different organic acids, which shows that the differences in solubility that are encountered at equilibrium are mainly explainable in terms of the solution chemistry of the various acids. These particular acids were tested because they either have already been used by previous workers in solubility studies, or else could possibly occur naturally in the environment of the tooth surface, viz. alanine, aspartic, citric, glutamic, lactic, malic, malonic, succinic and valine.

General principles

These organic acids are weak acids in the strict chemical sense, and exert a buffering action at any particular pH by an amount that is a function of the dissociation constant(s) of the acids. These acids have different dissociation constants, and so have buffering capacities different from each other at different pH's.

If no reaction other than acid decalcification takes place, the amount of mineral dissolved at equilibrium and the final pH of a particular acid solution at a particular initial pH is a function of the buffering capacity of the acid at that initial pH (LEACH, 1959).

It may then be presumed that at equilibrium the decalcification brought about by different acids at the same initial pH should correspond to the buffering capacities of the acid solutions and any deviations from this may then be presumed to be evidence that some other mechanism is also affecting the decalcification processes.

Solubility and pH

It is essential that the pH in all the solutions must initially be the same, as the solubility of the mineral matter is very sensitive to changes in pH. An empirical relationship for the solubility expressed in terms of calcium has been given by HODGE (1951) from solubility data collected from the literature. This may be expressed in the form $\log (Ca^{2+}) + pH = K$, where K is an empirical constant. From this relationship it is seen for example that a pH change of 0·3 pH units should change the solubility with respect to calcium twofold. Insufficient attention to this detail can easily lead to apparently discrepant results.

Rate of dissolution and equilibrium solubility

The rigorous dependence of the solubility on the buffering capacity is only applicable when the system has attained equilibrium. Before equilibrium has been reached the reaction is much more complicated, and is controlled by the diffusion rates of the various ions that are present in the liquid in the immediate environment

of the crystal surfaces (GRAY, 1958). There the pH and concentration of the various ions will be different from that of the bulk solution, in some cases being advantageous, and in others disadvantageous, to the decalcification processes.

MATERIALS AND METHODS

Enamel and dentine samples

The method was essentially that fully described elsewhere (LEACH, 1959) with the exception that the material was ground to a fine powder in a water-cooled micro-disintegrator so as to pass a 60 mesh sieve.

Acid buffer solutions

Each of the buffer solutions was made to pH 4·70 and contained 0·2 M sodium acetate–acetic acid buffer, 1 M sodium chloride to maintain a relatively constant ionic strength and one each of the acids under test, so that the acid and its various anions were present to a total concentration of 0·1 M. It is necessary to have a supporting acetate buffer as some of the acids are poor buffers and would produce little decalcification and large pH changes if used alone. A solution containing the acetate buffer and sodium chloride only was used as control.

Buffering capacity

The buffering capacity of each solution was estimated as the amount of 0·1 N NaOH required to change the pH of 10 ml of the original solutions from 4·70 to 5·00 (Table 1).

TABLE 1. MILLILITRES 0·1 N NaOH TO CHANGE THE pH OF 10 ml OF THE SOLUTIONS FROM 4·70 TO 5·00

Each solution contains 0·2 M sodium acetate-acetic acid, 1 M sodium chloride and one each of the following acids and their various anions to a total concentration of 0·1 M

Acid	0·1 N NaOH (ml)
Control	3·20
Alanine	3·20
Valine	3·20
Aspartic	3·68
Lactic	3·72
Glutamic	4·00
Malonic	4·10
Malic	4·90
Succinic	5·10
Citric	5·80

Decalcification

100 mg of enamel or dentine were shaken overnight in 10 ml of the appropriate solution at room temperature. The solutions were then centrifuged (5 min, R.C.F. 1200 g) and the supernatant solutions filtered through molecular filter membrane (pore size 470 mμ). The pH of the solutions was determined and analysed for calcium and phosphorus by the methods previously described (LEACH, 1960). Triplicate tubes of each solution were set up and these gave results that agreed to within 5 per cent of each other.

RESULTS AND DISCUSSION

In Fig. 1 is shown the relationship between the final pH of the various acid solutions after reaction with the powdered enamel and dentine and their experimental titratable acidities as described above. There is an inverse linear relationship between the final pH of the solutions and their respective buffering capacities when expressed in this manner.

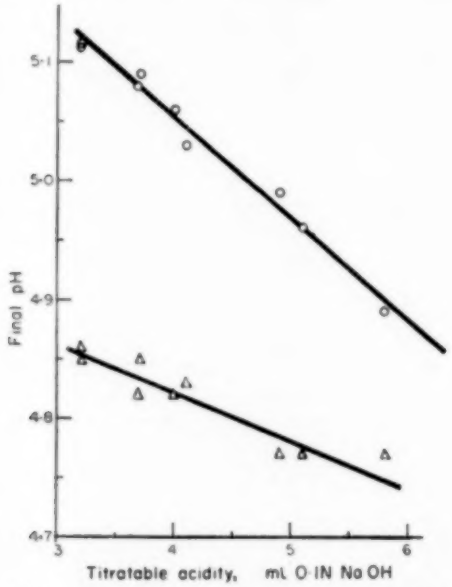


FIG. 1. Final pH of solutions of acids of different buffering capacities (initial pH 4·70) after shaking 100 mg of enamel (Δ) or dentine (o) in 10 ml of the solution overnight at room temperature.

In Figs. 2 and 3 are shown both the calcium and phosphorus released from the enamel and dentine respectively plotted against the buffering capacities of the acid solutions. With the exception of the points at the extreme right of each figure, which represent the results obtained with citric acid, there is a linear relationship

between the solubility and the buffering capacity of each acid solution. Citric acid is known to be taken up by the mineral matter of enamel and dentine, and by hydroxyapatites in general, in a complex manner to form a mineral of different

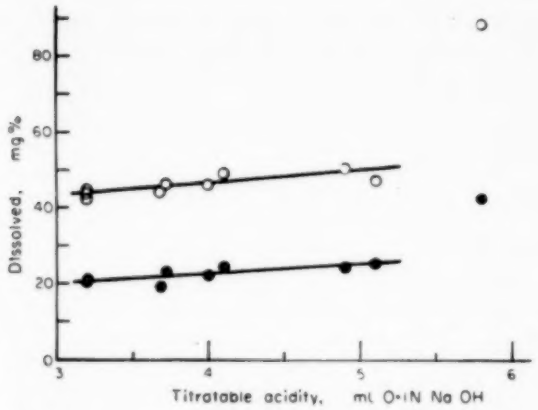


FIG. 2. Milligrams of Ca (o) and P (●) released (as mg per cent of solution) on shaking 1 g of enamel in 100 ml solutions of acids of different buffering capacities overnight at room temperature.

chemical properties than before, at the same time releasing excess phosphate from the crystal surfaces (NEUMAN and NEUMAN, 1958). Citric acid is thus not expected to follow a decalcification pattern that is solely dependent on the buffering capacity

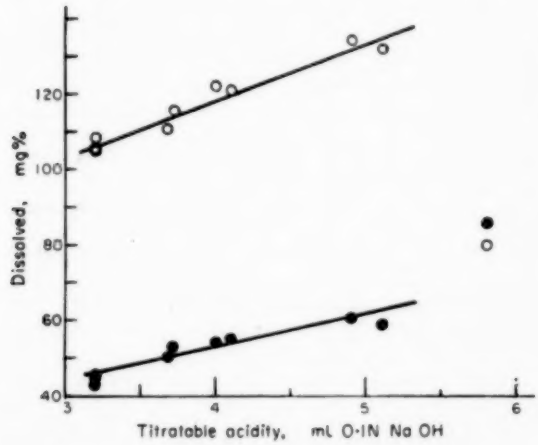


FIG. 3. Milligrams of Ca (o) and P (●) released on shaking 1 g of dentine in 100 ml solutions of acids of different buffering capacities overnight at room temperature.

of the solution. There is also the possibility that the various citrate ions in the solution react with the free calcium ions to form chelated or associated compounds, but it is not possible to detect their presence in the present instance because of the other reactions taking place. The other acids are also known to form various associated compounds with calcium in solution and their concentrations can be calculated approximately from the published stability constants in the literature (BJERRUM, SCHWARZENBACH and SILLÉN, 1957). The amounts so formed are calculated to be small in comparison to the total amount of calcium that is present in the solution as a result of the acid decalcifying action of the solutions and this is corroborated by the linear relationships found above.

There is no evidence from the present series that, with the exception of citric acid, any of the acids tested for their decalcifying ability are able to influence these processes by any mechanism other than that provided by the buffering capacities of the acid solutions.

Acknowledgements—Thanks are specially due to Miss A. M. DODGE for her outstanding technical assistance, to all members of the Dental School who assisted in the collection of teeth, and to the Medical Research Council for financial assistance.

REFERENCES

BJERRUM, J., SCHWARZENBACH, G. and SILLÉN, L. G. 1957. *Stability Constants, Part* I: *Organic ligands*. The Chemical Society, London.

BUONOCORE, M. 1958. Solubility of enamel, dentin and bone in acetate, lactate and aspartate buffers. *J. dent. Res.* **37**, 57.

BUONOCORE, M. G. and SOMMER, B. 1954. A study of the effect of the addition of certain amino acids to enamel-decalcifying buffers. *J. dent. Res.* **33**, 650–651.

BUONOCORE, M. G. and SOMMER, B. 1957. The effects of the addition of certain amino acids to enamel decalcifying buffers. *J. Amer. dent. Ass.* **55**, 781–784.

GRAY, J. A. 1958. Solubility rate of human dental enamel. *J. dent. Res.* **37**, 57–58.

HODGE, H. C. 1951. *Metabolic Interrelations*, p. 91. Josiah Macy Jr. Foundation, Third Conference, New York.

KOULOURIDES, T. and BUONOCORE, M. G. 1958. Effect of certain anions on the solubility of enamel and dentin. *J. dent. Res.* **37**, 57.

LEACH, S. A. 1959. Some notes on the solubility of enamel and dentine in acid. *Arch. oral Biol.* **1**, 218–232.

LEACH, S. A. 1960. Electrophoresis of synthetic hydroxyapatite. *Arch. oral Biol.* **3**, 48–56.

LITTLE, M. F., BUONOCORE, M. G. and SOMMER, B. 1954. Comparative study of the enamel decalcifying ability of different acids. *J. dent. Res.* **33**, 671.

MCCLURE, F. J. and RUZICKA, S. J. 1946. The destructive effect of citrate vs. lactate ions on rats' molar tooth surfaces *in vivo*. *J. dent. Res.* **37**, 57.

NEUMAN, W. F. and NEUMAN, M. W. 1958. *The Chemical Dynamics of Bone Mineral*. University of Chicago Press.

WYNN, W., HALDI, J. and LAW, M. L. 1960. Enamel demineralisation. *Dent. Progr.* **1**, 4–7.

Arch. Oral Biol., Special Supplement, Vol. 6. pp. 125–129, 1961. Pergamon Press Ltd.

THE STORAGE OF MOLYBDENUM

I. PORCSALMY*, P. ADLER† and Z. NAGY*

*Department of Medical Chemistry and †Department of Stomatology, University Medical School, Debrecen, Hungary

Abstract—Molybdenum levels were estimated in the liver, kidney, and spleen of three generations of albino rats consuming water with 0·1 p.p.m. Mo. Compared with controls (consuming distilled water), the first generation showed no differences in these three parenchymatous organs. In the liver and kidney in both the second and third and in the spleen in the third generation, higher levels of Mo were found.

Zusammenfassung—Der Molybdängehalt wurde in der Leber, Niere und Milz von drei Rattengenerationen bestimmt, deren Trinkwasser 0,1 p.p.m. Mo enthielt. Im Vergleich zu Kontrollen (die dest. Wasser tranken) war der Mo-Gehalt der drei genannten parenchymatösen Organen in der ersten Generation unverändert; in der Leber und Niere haben wir in der zweiten, in der Milz erst in der dritten Generation einen erhöhten Molybdängehalt festgestellt.

Vol. 6
1961

IN PREVIOUS experiments the caries-protective effect of traces of water-borne molybdenum was demonstrated in the rat (ADLER, 1951; ADLER and PORCSALMY, 1960), without having clarified its mode of action. Our knowledge of the role of molybdenum in the metabolism is rather limited. Xanthin-oxidase is known to contain it in considerable concentration; a competitive antagonism is assumed to exist between Mo and Cu ions. Nothing is known, however, about the excretion, storage or availability in metabolic processes of ingested molybdenum in the rat organs. In order to throw some light upon these problems in the rats—used in long term caries experiments by ADLER and PORCSALMY— molybdenum levels were estimated in the liver, kidneys, and spleen as these are sites of important metabolic activity.

METHODS

The planning and duration of the experiments and the number of animals used, has been described in an earlier publication (ADLER and PORCSALMY, 1960). When the animals were sacrified at the termination of the experiments, livers, kidneys and spleens were removed from the cadavers immediately. The respective organs of animals living in the same cage were pooled in glass stoppered bottles and were kept in a refrigerator until used for analytical procedures. Although at the start of each experimental periode, four animals lived in each cage, not all survived the total experimental period. The Mo level was ascertained in the aforementioned organs of the survivors only. As the respective organs of survivors from the same cage were pooled, our analytical results do not refer to individual animals but to

the pooled organs. In most experiments, the pooled organs of survivors from three cages were used, in a few, however from two cages only.

Estimation of molybdenum was carried out by emission spectral analysis after digestion of the pooled organs in a mixture of concentrated nitric acid and hydrogen peroxide. In the dried anorganic remnants of this destruction, we were unable, however, to detect molybdenum directly; therefore an enrichment procedure was adhered to: an aliquot of the digested acid mixture (corresponding to 1 g liver substance, to 5 g kidney substance, and 5 g spleen substance) was neutralized with liquid ammonia, the volume being filled up with redistilled water to 20 ml. To this neutralized mixture 3 ml of a 2·5% EDTA solution was added in order to form complexes with several metals. By adding 20% doubly distilled HCl, pH was regulated to approximately 1·0. Then 0·5 ml of a 0·33% morin solution* (dissolved in butanol), and 5 ml pure butanol were added to the mixture which was afterwards thoroughly shaken for 1 min. With the watery phase the butanol extraction was then twice repeated. The material from the three butanol extractions was handled thereafter together. In this extract, besides molybdenum, the following metals occur: Ti, Sn, Sb. They do not cause, however, any disturbance of the spectral procedures for molybdenum estimation.

After having expelled butanol from the morin–butanol extract, the solid remnant was incinerated in an electric furnace, at approximately 550°C. The incinerated material was then dissolved in two drops of 20% metal-free hydrochloric acid. As a spectrographic buffer, 1 drop of 5% KCl was then added to it. Thereafter, the material was dried again at 100°C. Finally, the solid was dissolved in one drop redistilled water, and taken up quantitatively into a glass capillary. With this watery solution, according to SCHEIBE and RIVAS' method (1936), the one carbon electrode was imbibed.

In preparing spectrographic standards, known quantities of molybdenum—in the range of the expected concentrations in experimental samples—were added to the remnants of the rat organs after the total extraction of molybdenum as described above. With these, the extraction procedures were carried out again; the extracts obtained in this way were used for constructing calibration curves.

A Q 24 Zeiss (quartz) spectrograph was used, with UV light, adhering to the logarithmic wedge sector method. Excitation was carried out in an alternating current arc, under controlled electric conditions. Photographic records were made in every instance, their evaluation being based upon the length of the most sensitive molybdenum lines (wave lengths 3133, 3170 and 3197 Å, respectively). In this way, as a minimum approximately 1·5 μg of molybdenum can be detected; our calibration curves were prepared, however, for ranges between 3 and 30 μg.

* Morin is a flavon dye.

With regard to the rather intricate preparative procedures, a considerable error may be inherent in our estimations, not exceeding, however, 25–30 per cent. The method used we consider, therefore, as a semiquantitative one. This fact should be taken into account when evaluating our findings.

RESULTS

Molybdenum levels in the liver, kidney and spleen of controls, and experimental animals, from the parental, first and second filial generations are summarized in Table 1. Molybdenum levels are expressed in μg/100 g wet wt. In all three generations, experimental animals consumed water with 0·1 p.p.m. Mo, at pH 6, 7 and 8, respectively.

TABLE 1. LEVELS OF MOLYBDENUM IN THE LIVER, KIDNEY AND SPLEEN IN μg/100 g WET ORGAN WT.

Generation and group	Liver	Kidney	Spleen
A. Parental generation			
Control	25·0±2·65	14·0±1·22	8·3±1·00
0.1 p.p.m. Mo			
pH 6·0	26·3±3·54	13·7±1·58	6·9±1·10
pH 7·0	27·0±3·81	15·0±2·35	8·9±1·10
pH 8·0	24·3±2·17	13·0±1·73	8·3±0·80
B. First filial generation			
Control	22·7±1·58	14·7±0·70	7·7±0·70
0·1 p.p.m. Mo			
pH 6·0	40·3±3·68	18·0±1·75	9·8±1·30
pH 7·0	54·7±2·55	19·3±1·73	13·2±0·95
pH 8·0	47·3±5·70	19·0±2·00	7·6±1·00
C. Second filial generation			
Control	29·3±3·68	14·7±1·00	7·2±0·70
0·1 p.p.m. Mo			
pH 6·0	99·0±4·95	37·0±4·12	15·0±1·59
pH 7·0	178·0±8·66	46·0±2·35	23·3±3·87
pH 8·0	116·0±6·25	27·0±5·29	20·0±0·71

From the data of Table 1, it can be concluded that:

(1) Between the Mo-levels in the organs of controls of the three generations no conspicuous differences were found.

(2) In some instances, within the same generation considerable differences occur in the Mo-levels of the same organ, and are related to the pH of the drinking water. In the kidneys as well as in the spleens and livers, the highest levels were found associated with pH 7 in the drinking water. In the spleen the differences are more marked in the first filial generation; in the kidney, on the other hand, they become manifest in the second filial generation only. In the liver the difference in the second filial generation is more pronounced than in the first.

(3) In all experiments, in which the pH of the drinking water was the same for the three generations, a marked increase occurs in the molybdenum levels in the later offspring.

DISCUSSION

Due to the semiquantitative nature of the molybdenum estimations, no special significance can be attributed to differences within the same generation arising from the different pHs of the drinking water. Our findings suggest that this factor influences the storage of molybdenum, but in order to settle this problem definitely, more material is required.

Since higher levels were found in the offspring than in the parents, and no change occurred in the Mo-levels of the controls, it is justifiable to form of three generations of controls one group, and of each generation of experimental animals a second, third and fourth group, without distinguishing differences in pH of the drinking water. In this simplified way, figures of Table 2 were obtained. From these figures the statistical significance of the increased molybdenum levels in the later genera-

TABLE 2. SUMMARIZED FINDINGS ON LEVELS OF MOLYBDENUM IN THE LIVER, KIDNEY AND SPLEEN, IN THREE GENERATIONS OF RATS, RECEIVING 0·1 p.p.m. MOLYBDENUM IN THE DRINKING WATER (EXPRESSED IN μg/100 g WET ORGAN WT.)

Group	Liver	Kidney	Spleen
Controls of all three generations	25·64±1·46	14·4±0·70	7·77±0·37
	Not significant	Not significant	Not significant
Parental generation pH 6, 7 and 8	25·90±1·70	13·9±1·73	8·04±0·18
	Significant	Significant	Not significant
First filial generation pH 6, 7 and 8	47·40±3·00	18·8±0·77	10·02±1·12
	Significant	Significant	Significant
Second filial generation pH 6, 7 and 8	131·00±12·49	36·8±3·32	19·40±1·80

tions of the experimental animals is obvious. In no instance does a significant difference occur between controls and the parental generation. In the liver, and in the kidneys, the difference between the parental and first filial generation is statistically significant; in the spleen the difference is not significant. On the other hand, the second filial generation has higher molybdenum levels than the first one in the spleen too.

No inference can be drawn from our results as to the metabolic role of molybdenum. Neither can we draw conclusions as to the mode of its caries-protective action. The higher levels of Mo in these three organs do not display any correlation with the caries figures of the same groups. That higher levels were found after longer exposure in the first filial than in the parental generation of experimental animals, is not surprising. Some importance may be attributed, however, to the considerably higher levels of Mo in the second filial generation in comparison to the first one, in spite of a markedly shorter exposure period. This may be evidence of some role of molybdenum in the rat's metabolism. Then was no evidence in the second filial generation of our experimental animals to indicate a toxic effect of this increased Mo storage in the aforementioned parenchymatous organs.

Vol. 6
1961

REFERENCES

ADLER, P. 1957. Experiments with albino rats upon the caries-protective effect of water-borne molybdenum. Proc. IVth ORCA Congress, pp. 48–53.

ADLER, P. and PORCSALMY, I. 1960. Neuere Versuche über den karies-protektiven Effekt des im Trinkwasser enthaltenen Molybdäns. Verh. VII. Tagg. ORCA, pp. 193–198 (*Arch. oral Biol.* **4**).

SCHEIBE, G. and RIVAS, A. 1936. Physical methods in the chemical laboratory—XXXII. A new method of quantitative emission spectrum analysis which can also be used as a micromethod. *Angew. Chem.* **49**, 443–446 (*Chem. Abstr.* **30**, 6302).

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 130–133, 1961. Pergamon Press Ltd.

CITRATE IN BONE AND DENTINE

R. L. HARTLES

School of Dental Surgery, University of Liverpool, England

Abstract—Ox bone and human dentine contain nearly 1 per cent of citrate. A portion of the citrate seems to be associated with a peptide. The normal rat femur contains 0·53 per cent citrate. On a diet containing adequate calcium (0·56 per cent) but no vitamin D, the citrate content of the femur is depressed slightly. In the presence of excessive amounts of vitamin D the bone citrate is almost doubled. On a calcium-deficient diet (0·03 per cent) and in the absence of vitamin D the bone citrate is only half the normal value. When the intake of the vitamin is normal and the calcium intake remains low, the bone citrate increases (0·7 per cent). Excess of the vitamin again causes a large increase in bone citrate. The amount of citrate in bone seems to be governed by the vitamin D intake of the animal, but the effects of a deficiency of the vitamin can be mitigated by an adequate intake of calcium. On calcium deficient diets the bone is poorly formed both in the presence and absence of vitamin D, but the animals develop tetany only in the absence of vitamin D. The major chemical difference caused by vitamin D is an increase in bone citrate.

Zusammenfassung—Ochsenknochen und menschliches Dentin enthalten beinahe 1% Zitrat. Ein Teil des Zitrates scheint an einem Peptid zu hängen. Der normale Rattenfemur enthält 0,53% Zitrat. Bei einem Futter, das genügend Calcium (0,56%) aber kein Vitamin-D enthält, sinkt der Zitratgehalt des Femurs etwas. Bei extremem Vitamin-D-Gehalt wird der Zitratgehalt im Knochen beinahe verdoppelt. Bei einem calciumarmen Futter (0,03%) ohne Vitamin-D ist der Zitratgehalt des Knochens nur die Hälfte vom Normalen. Wenn die Vitamin-D-Aufnahme normal, dagegen die von Calcium niedrig ist, steigt der Zitratgehalt des Knochens (0,7%). Ein Überschuß von Vitamin verursacht dagegen ein Ansteigen des Knochenzitrates. Der Zitratgehalt des Knochens scheint durch die Vitamin-D-Aufhahme des Tieres gesteuert zu werden, aber die Auswirkungen des Vitaminmangels können durch genügende Calciumzufuhr gemildert werden. Bei Calciummangelfutter wird der Knochen mit oder ohne Vitamin-D minderwertig ausgebildet, dagegen entsteht eine Tetanie bei den Tieren nur ohne Vitamin-D. Der hauptsächliche chemische Unterschied, der von Vitamin-D hervorgerufen wird, ist ein Ansteigen des Knochenzitrates.

INTRODUCTION

CITRATE appears to be present in all mammalian mineralized tissues. DICKENS (1941) showed that the ribs and leg bones, including marrow, of the kitten contained nearly 0·4 per cent of citrate; cartilage from the same animals contained only 0·03 per cent. The citrate seems therefore to accompany the deposition of the bone mineral. The precipitation of any form of basic calcium phosphate in a medium which contains citrate results in the coprecipitation of the citrate. ARMSTRONG and SINGER (1956) have expressed the view that the presence of citrate in the skeleton is adventitious

and that citrate accumulates in the skeleton because hydroxyapatite has a great affinity for citrate at physiological hydrogen ion concentrations. Other workers are less convinced that the changes which can be demonstrated in the bone citrate as a result of nutritional disturbance are explicable in this way.

THE OCCURRENCE OF CITRATE IN BONE AND DENTINE

Only a small fraction (5–15 per cent) of the total citrate in powdered bone or dentine can be extracted with either cold or hot water (FREE, 1943; HARTLES and LEAVER, 1960). These observations suggest that the citrate does not exist solely as calcium citrate. Evidence has been obtained recently (HARTLES and LEAVER, 1960; LEAVER, EASTOE and HARTLES, 1960) to show that some of the bone and dentine citrate may be associated with a peptide. Such an association, if substantiated, suggests that not all of the citrate present is of adventitious origin.

Vol. 6
1961

INFLUENCE OF NUTRITIONAL STATUS ON THE BONE CITRATE IN THE RAT

The normal rat femur contains about 0·53 per cent citrate (NICOLAYSEN and EEG-LARSON, 1956; HARTLES and LEAVER, 1961 a,b,c). Results have been reported which show bone citrate is governed by both calcium and vitamin D intake (STEENBOCK and BELLIN, 1953; CARLSSON and HOLLUNGER, 1954). NICOLAYSEN and EEG-LARSON (1956) believe that although both calcium and vitamin D are necessary for citrate production in bone, the effects of lack of the vitamin can be ameliorated by an adequate intake of calcium and phosphorus.

The exact nature of the relationship between bone citrate, calcium and phosphorus metabolism, and vitamin D intake is still obscure. In an attempt to gain information on this relationship HARTLES and LEAVER (1961a,b,1962) used purified diets containing either normal (0·56 per cent) or low (0·03 per cent) calcium, normal (0·42 per cent) or low (0·06 per cent) phosphorus and low, normal or high contents of vitamin D. In order to gain some degree of uniformity, their analyses were carried out on that portion of the rat femur of s.g. not less than 1·5. This is an arbitrary division but has some merit in that it excludes non-mineralized soft tissues from the analysis. The results are presented in Tables 1 and 2.

In the normal rat receiving a diet adequate in minerals and vitamin D the femoral bone contains 0·53 per cent of citrate. When the animal was deprived of vitamin D the bone citrate fell slightly but significantly to 0·46 per cent. If the diet contained excessive amounts of vitamin D (250 times the normal level) the bone citrate was raised to 0·96 per cent. Thus in these circumstances there is a positive correlation between the dietary level of vitamin D and the citrate content of bone.

When the diet is low in calcium a deficiency of the vitamin results in a very low bone citrate (0·24 per cent), a normal vitamin intake raises the bone citrate to 0·68 per cent, excess of the vitamin raises the bone citrate to 0·97 per cent. Again a positive correlation between dietary vitamin D and bone citrate.

If the diet is low in phosphorus the results are rather different. A deficiency of vitamin D appears to cause an elevation of the bone citrate to 0·8 per cent, a figure which is unaltered in the bone of animals receiving adequate dietary vitamin D. When the dietary content of the vitamin is excessive the bone citrate again rises to about 1 per cent. With an adequate dietary supply of phosphorus, the citrate content of bone increases with increasing dietary content of vitamin D. This relationship is much less apparent on diets low in phosphorus but adequate in calcium.

TABLE 1. THE CITRATE CONTENT (% W/W) OF THE RAT FEMUR AT VARYING LEVELS OF VITAMIN D INTAKE AND AT LOW AND NORMAL INTAKE OF PHOSPHORUS AND CALCIUM

Dietary		Citrate % in rat femur		
		Vitamin D in diet		
P(%)	Ca(%)	None	Normal	High
0·42	0·56	0·46	0·53	0·96
0·06	0·56	0·80	0·80	1·00
0·42	0·03	0·24	0·68	0·97

TABLE 2. THE CITRATE/CALCIUM RATIO IN THE RAT FEMUR AT VARYING LEVELS OF VITAMIN D INTAKE AND AT LOW AND NORMAL INTAKE OF PHOSPHORUS

Dietary		mg Citrate/g calcium in rat femur		
		Vitamin D in diet		
P(%)	Ca(%)	None	Normal	High
0·42	0·56	19·5	21·6	41·2
0·06	0·56	42·1	41·0	49·9
0·42	0·03	12·2	34·0	47·7

The accumulation of citrate in the rat femur therefore seems to depend on vitamin D, the highest amounts of citrate are found when the vitamin intake is excessive even when intakes of calcium or phosphorus are very low. But the effects of vitamin deficiency on bone citrate can be mitigated if the diet contains adequate calcium, particularly when the phosphorus intake is low.

REFERENCES

ARMSTRONG, W. D. and SINGER, L. 1956. In: *Ciba Foundation Symposium on Bone Structure and Metabolism.* Churchill, London.

CARLSSON, A. and HOLLUNGER, G. 1954. *Acta. physiol. scand.* **31**, 317.

DICKENS, F. 1941. *Biochem. J.* **35**, 1011.

FREE, A. 1943. *J. dent. Res.* **22**, 477.

HARTLES, R. L. and LEAVER, A. G. 1960. *Arch. oral Biol.* **1**, 297.
HARTLES, R. L. and LEAVER, A. G. 1961a. *Arch. oral Biol.* **5**, 38.
HARTLES, R. L. and LEAVER, A. G. 1961b. *Arch. oral Biol.* **5**, 214.
HARTLES, R. L. and LEAVER, A. G. 1962. *Arch. oral Biol.* In press.
LEAVER, A. G., EASTOE, J. E. and HARTLES, R. L. 1960. *Arch. oral Biol.* **2**, 120.
NICOLAYSEN, R. and EEG-LARSON, N. 1956. In: *Ciba Foundation Symposium on Bone Structure and Metabolism.* Churchill, London.
STEENBOCK, H. and BELLIN, S. A. 1953. *J. biol. Chem.* **205**, 985.

Vol. 6
1961

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 134–138, 1961. Pergamon Press Ltd.

TRANSAMINASEN IM MENSCHLICHEN SPEICHEL

A. KRÖNCKE

Zahnärztliches Institut der Universität Tübingen, Deutschland

Zusammenfassung—Im Nüchternspeichel kariesanfälliger und kariesresistenter Personen wurde die Aktivität der Glutamat-Oxalacetat-Transaminase (GOT) bestimmt. Dabei wurde folgendes beobachtet:

(1) Die GOT-Aktivität ist im menschlichen Nüchternspeichel mit Werten bis zu 163 GOT-Einh./ml (WROBLEWSKI) im allgemeinen wesentlich höher als im Serum.

(2) Im Speichel kariesresistenter Personen ist die GOT-Aktivität größer (x= 107,8 GOT-Einh./ml) als bei Kariesanfälligen (x=44,5 GOT-Einh./ml). Die Gruppendurchschnitte unterscheiden sich signifikant (t=5,99).

(3) Die GOT-Aktivität wächst proportional zum Proteingehalt des Speichels, nicht jedoch in gleichem Ausmaß wie die Speichelatmung. Sie wird daher wahrscheinlich nicht von den gleichen Faktoren beeinflußt, die für den charakteristisch verlangsamten Glukoseabbau im Speichel Kariesanfälliger verantwortlich sind.

Abstract—The activity of glutamic-oxalacetic-transaminase (GOT) was evaluated in fasting saliva samples of caries-susceptible and caries-resistant persons. The results are:

(1) Generally, the GOT-activity in human saliva is higher (up to 163 GOT-units/ml (WROBLEWSKI) than it is in human serum.

(2) In fasting saliva samples of caries-resistant persons the GOT-activity is higher (x=107·8 GOT-units/ml) than it is in such of caries-susceptible persons (x=44·5 GOT-units/ml). The mean values of both groups differ significantly (t=5·99).

(3) The GOT-activity increases proportionally to the protein content of the saliva samples, but not in the same rate as the respiration. Therefore, the GOT-activity in saliva seems not to be influenced by the same factors which cause the characteristic reduced glucose degradation in saliva of caries-susceptible persons.

EINLEITUNG

SEIT BRAUNSTEIN und KRITZMANN (1937) erstmals Stoffwechselprozesse beschrieben, bei denen Aminogruppen von Glutamin- oder Asparaginsäure auf α-Ketosäuren übertragen werden, beanspruchen diese Transaminierungsvorgänge auf den verschiedensten Forschungsgebieten grosses Interesse. Auch in der Klinik hat die Aktivitätsbestimmung der diese Reaktionen katalysierenden Fermente, der Transaminasen, für die Frühdiagnostik bestimmter pathologischer Zustände Bedeutung erhalten (LA DUE, WROBLEWSKI und KARMEN, 1954).

Im Rahmen der Kariesforschung wurde die Aufmerksamkeit durch die Untersuchungen von ATKINSON und MATHEWS (1949) sowie von ARMSTRONG und ATKINSON (1951, 1953) auf die vermehrte Anwesenheit von Glutaminsäure und Aspara-

Vol. 6
1961

ginsäure im kariösen Dentin und auf die Aktivität von Transaminasen gelenkt. SPRETER VON KREUDENSTEIN (1960) konnte später nachweisen, daß Transaminasen stets und in dem Serum ähnlichen Konzentrationen im "Dentinliquor" enthalten sind.

Auch im menschlichen Speichel wurden wie in anderen Körperflüssigkeiten Transaminasen nachgewiesen (PELLEGRINI, 1957; DREIZEN *et al.*, 1959) und auf eventuelle Beziehungen der Aktivität dieser Fermente zu pathologischen Vorgängen in der Mundhöhle untersucht (MARTINELLI und PELLEGRINI, 1957; BREUSTEDT, 1961), ohne allerdings eindeutige Zusammenhänge z.B. mit der Karies aufzudecken. Es ist möglich, daß die schwierige Beurteilung der klinischen Kariesanfälligkeit bzw. -resistenz für diese negativen Ergebnisse mitverantwortlich ist (KRÖNCKE, 1961a,b).

Uns interessierten die Transaminasen im Speichel kariesanfälliger und kariesresistenter Personen, weil diese Fermente bekanntlich zwei wichtige Metaboliten des Kohlenhydratstoffwechsels (Brenztraubensäure und α-Ketoglutarsäure) als Substrate besitzen und insofern eine wichtige Verbindung zum Eiweißstoffwechsel herstellen. Dabei darf der Glukoseabbau im Speichel und seine Beziehungen zur Karies als gut bekannt und weitgehend durchforscht gelten (LUNDQVIST, 1952; KRÖNCKE, 1959, 1960; NAUJOKS *et al.*, 1961; u.a.). Die Bedeutung dieser Verbindung zwischen Kohlenhydrat- und Eiweißstoffwechsel wird durch Versuche mit radioaktiv markiertem N^{15} offenbar, das in Eiweißkörper der Versuchstiere eingebaut und in allen darin vorkommenden Aminosäuren wiedergefunden wurde (EDLBACHER und LEUTHARDT, 1954). Dies zeigt, daß die Transaminasen im Mittelpunkt komplizierter Stoffwechselvorgänge stehen und daß ihre Wirkung keineswegs etwa mit den Produkten Glutaminsäure, Asparaginsäure oder Alanin beendet ist.

Aus diesem Grunde sollte geprüft werden, ob Unterschiede der Transaminaseaktivität im Speichel kariesanfälliger und kariesresistenter Personen und gegebenenfalls Zusammenhänge mit dem Kohlenhydratabbau im Speichel zu beobachten sind.

MATERIAL UND METHODEN

Untersucht wurden Nüchternspeichelproben von 24 kariesanfälligen (kA), 21 kariesresistenten (kR) und 6 als fraglichkariesanfällig (k?) beurteilten Personen. Der Grad der Kariesanfälligkeit wurde dabei klinisch nach einjähriger zahnärztlicher Beobachtung und nach dem Ergebnis eines experimentellen Kariesanfälligkeitstestes (Speichelatmung) bestimmt. Bei einem großen Teil dieser Versuchspersonen lagen auch Messungen der Pufferkapazität des Nüchternspeichels vor (KRÖNCKE 1961a), deren Ergebnisse in Zweifelsfällen zusätzlich herangezogen wurden.

Die Aktivität der Glutamat-Oxalacetat-Transaminase (GOT) im Speichel wurde in Anlehnung an KARMEN, WROBLEWSKI und LA DUE (1955) mit Hilfe der Fermenttestkombination TCA 15 971 der Fa. Boehringer, Mannheim bestimmt, das Untersuchungsverfahren dabei wie folgt modifiziert:

Frischer Nüchtern-Ruhespeichel wurde 1:1 mit Phosphat-Asparaginat-Puffer verdünnt. Da im zellhaltigen Speichel DPNH-verbrauchende Reaktionen weiterlaufen, kommt anders als bei der Bestimmung im Serum kein Gleichgewicht zwischen DPN und dem photometrisch bei 366 nm zu messendem DPNH zustande. Deshalb wurde der Extinktionsabfall während der Inkubation (15′) und nach Zugabe des Substrats (α-Ketoglutarat) gemessen und die Differenz zwischen beiden zur Berechnung der GOT-Aktivität eingesetzt.

In Anlehnung an LA DUE *et al.* (1954) geben wir die Transaminaseaktivität in gleichen Einheiten wie für Serum an.

Berechnung: $E^{366}_{1\ min} \cdot 4000 \cdot 1{,}89$ = GOT-Einheiten/1 ml Speichel.

ERGEBNISSE

Über die Ergebnisse unserer Transaminasebestimmungen gibt—nach Kariesanfälligkeit und Kariesresistenz der untersuchten Personen aufgeteilt—die folgende Tabelle 1 Auskunft:

TABELLE 1

Klinische Beurteilung	kA	k ?	kR
Kariesanfälligkeitstest (Speichelatmung/l ml/Std) (μl)	<65	66–90	>90
Anzahl der Messungen	24	6	21
Speichelatmung (μl O_2/Std.) $\bar{x}$ =	43	76	138
$s_{\bar{x}}$ =	3,16	3,1	9,4
GOT-Aktivität $\bar{x}$ =	44,5	91,9	107,8
$s_{\bar{x}}$ =	5,16	16,5	9,64
t =	2,87	5,99	0,80
P =	<1	<0,1	>5

Hieraus geht hervor, daß die durchschnittliche GOT-Aktivität im Speichel kariesresistenter Personen mit signifikanten Differenzen größer ist als diejenige im Speichel Kariesanfälliger. Innerhalb beider Gruppen fallen nur wenige Einzelwerte aus der Reihe, wie auch die relativ niedrigen Werte für die Standardabweichungen der Mittelwerte ($s_{\bar{x}}$) erkennen lassen.

Vol. 6
1961

DISKUSSION DER ERGEBNISSE

Übereinstimmend mit den Befunden von DREIZEN *et al.* (1959) wurden im menschlichen Nüchternspeichel GOT-Aktivitäten beobachtet, die mit Werten bis zu 163 Einheiten/ml erheblich größer als die im Serum vorkommenden (15–40 GOT-Einheiten/ml) sind.

Die Speichelproben Kariesanfälliger unterscheiden sich auch bezüglich ihrer Transaminaseaktivität in ähnlicher Weise von denen Kariesresistenter, wie dies von der Speichelatmung oder von der Aldolase-Aktivität her bekannt ist (NAUJOKS *et al.*, 1961). Ursprüngliche arbeitshypothetische Vermutungen, daß der bei Kariesanfälligen verringerte Sauerstoffverbrauch des Speichels womöglich durch vermehrte Aufnahme von Glukoseabbauprodukten in eine gesteigerte Eiweissynthese erklärbar sei, können daher nicht zutreffen. In diesem Falle hätte die GOT-Aktivität in der kA-Gruppe größer sein müssen als in der kR-Gruppe.

Bei der Betrachtung der Durchschnittswerte (x) fällt auf, daß die Differenz zwischen den Gruppen kA und kR bei der Speichelatmung nicht unerheblich größer ist als bei den Durchschnittswerten der GOT-Aktivität. Diese Differenz läßt sich sinnfällig als Relation ausdrücken, bei der der für kA geltende Wert = 1 gesetzt wird (siehe Tabelle 2). Zum Vergleich werden zwei nach Ergebnissen von NAUJOKS *et al.* (1961) berechnete Daten für die durchschnittliche Speichelatmung sowie für den Proteingehalt des Speichels annähernd gleichgroßer kA und kR-Probandengruppen angeführt.

TABELLE 2. VERGLEICH DER SPEICHELATMUNG, DER GOT-AKTIVITÄT UND DES PROTEINGEHALTES KARIESANFÄLLIGER (kA) UND KARIESRESISTENTER (kR) PROBANDENGRUPPEN.
DIE MIT* VERSEHENEN ANGABEN SIND DEN BEFUNDEN VON NAUJAOKS *et al.* (1961) ENTNOMMEN, DIE MIT† VERSEHENEN DATEN ENTSPRECHEN DEN VORLIEGENDEN ERGEBNISSEN

	kA : kR
Speichelatmung†	43 : 138 = 1 : 3,2
Speichelatmung*	43 : 156 = 1 : 3,6
GOT-Aktivität†	45 : 108 = 1 : 2,4
Protein-Gehalt*	7,6: 19,7 = 1 : 2,6

Diese Aufstellung läßt erkennen, daß die Speichelatmung Kariesresistenter gegenüber derjenigen Kariesanfälliger um mehr als um denjenigen Faktor größer ist, um den sein Proteingehalt durchschnittlich zunimmt. Dies entspricht auch der von uns früher festgestellten Beobachtung, daß die Speichelatmung zwar von den zelligen Elementen abhängig ist, jedoch außerdem durch ein im zellfreien Speichel enthaltenes Prinzip beeinflußt wird (BRAMSTEDT, 1957). Die GOT nimmt dagegen durchschnittlich nur um nahezu den gleichen Faktor zu wie der Proteingehalt. Man kann

daraus einerseits folgern, daß die GOT-Aktivität im Speichel dem Proteingehalt und damit in großen Grenzen der Zellzahl proportional ist. Dem entsprechen auch ähnliche Beobachtungen von BREUSTEDT (1961) und die Tatsache, daß reine Drüsenspeichelsekrete keine GOT-Aktivität aufweisen. Andererseits zeigt die Gegenüberstellung, daß die GOT im menschlichen Speichel offensichtlich nicht von den gleichen Faktoren beeinflußt wird, die wie beim Glukoseabbau Fermentaktivität und Stoffdurchsatz begrenzen und in irgendeiner Weise mit der Karies koordiniert sind.

Anmerkung—Diese Untersuchungen wurden durch Sachbeihilfen der Deutschen Forschungsgemeinschaft und der Research Corporation, New York, in dankenswerter Weise unterstützt.

LITERATUR

ARMSTRONG, W. G. und ATKINSON, H. F. 1951. Transamination in the human tooth. *Nature, Lond.* **168**, 695.

ARMSTRONG, W. G. und ATKINSON, H. F. 1953. An investigation into the acidic components of carious dentine. *Brit. dent. J.* **95**, 8.

ATKINSON, H. F. und MATHEWS, E. 1949. An investigation into the organic components of the human tooth. A study of sound and carious dentine. *Brit. dent. J.* **86**, 167–171.

BRAMSTEDT, F., KRÖNCKE, A. und NAUJOKS, R. 1957. Über den Einfluß von Fluor auf den Glukoseabbau isolierter Mundbakterienstämme. *Odont. Revy* **8**, 201–206.

BREUSTEDT, A. 1961. Die Glutaminsäure-Oxalessigsäure-Transaminase im menschlichen Speichel und ihre Bedeutung. *Dtsch. zahnärztl. Z.* **16**, 207.

DREIZEN, S., STONE, R. E., DREIZEN, J. G. und SPIES, T. D. 1959. Comparison of Glutamic-oxalacetic and Glutamic-pyruvic transaminase concentrations in human saliva and serum. *Proc. Soc. exp. Biol., N. Y.* **102**, 449–451.

EDLBACHER, S. und LEUTHARDT, F. 1954. *Lehrbuch der Physiologischen Chemie*. 11. Aufl., p. 358. De Gruyter, Berlin.

KARMEN, A., WROBLEWSKI, F. und LA DUE, J. S. 1955. Transaminase activity in human blood. *J. clin. Invest.* **34**, 126–133.

KRÖNCKE, A. 1959. *Freie Zucker im menschlichen Speichel und deren Beziehungen zur Zahnkaries*. Barth, Leipzig.

KRÖNCKE, A. 1960. Ergebnisse und Probleme biochemischer Speichelforschung. *Dtsch. zahnärztl. Z.* **14**, 523–528.

KRÖNCKE, A. 1961. Kariesteste auf der Grundlage von Speicheluntersuchungen. *Dtsch. zahnärztl. Z.* **16**, 185–188.

KRÖNCKE, A. 1961b. Diskussion zum Vortrag von A. BREUTSEDT. *Dtsch. zahnärztl. Z.* **16**, 207.

LA DUE, J. S., WROBLEWSKI, F. und KARMEN, A. 1954. Serum glutamic oxaloacetic transaminase activity in human acute transmural myocardial infection. *Science* **120**, 497–499.

LUNDQVIST, C. 1952. Oral sugar clearance. *Odont. Revy* **3**, Suppl 1.

MARTINELLI, G. und PELLEGRINI, R. 1957. L'attività transaminasica della saliva in alcune condizioni patologiche del cavo orale. *Boll. Soc. ital. biol. sper.* **33**, 72–74.

NAUJOKS, R., BRAMSTEDT, F., RANKE, E. und AUERBACH, M. 1961. Zur Problematik der Speichelgewinnung für biochemische Untersuchungen. *Dtsch. zahnärztl. Z.* **16**, 171–175.

PELLEGRINI, R. 1957. Prime reicerche sull'attività transaminasica della saliva. *Boll. Soc. ital. biol. sper.* **33**, 70–72.

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 139-148. 1961. Pergamon Press Ltd.

EXAMEN DES RÉPLIQUES DE LA SURFACE DE L'ÉMAIL AU MOYEN DU MICROSCOPE À CONTRASTE INTERFÉRENTIEL

SYSTÈME NOMARSKI

L. GRIMBERT* et Mlle G. PIGEAT†

*École Dentaire de Paris et †Institut d'Optique

Vol. 6
1961

Résumé—Les études de la surface de l'émail au moyen des répliques ont montré qu'elles doivent porter sur un nombre considérable de dents et qu'il convient de leur donner une forme statistique. Dans ces conditions, une technique permettant de supprimer un temps opératoire aussi délicat que l'ombrage des répliques aura un réel intérêt.

Le microscope à contraste interférentiel (système Nomarski) permet un examen direct sans ombrage de ces répliques. Pour interpréter les images contrastées obtenues et connaître la nature exacte du relief, il faut déterminer le sens de la lumière tangentielle. Ceci s'obtient aisément par comparaison.

De nombreux examens ainsi pratiqués ont permis de préciser certains points controversés. Des expériences permettent d'affirmer que les périkymaties sont créés par la fusion de plusieurs lignes de Retzius et expliquent les qualités de dureté et de résistance aux acides de la surface.

Les érosions ne sont pas recouvertes de cuticule et les causes premières de leur formation sont surtout mécaniques. Les dents humaines de l'époque néolithique montrent la présence de cuticule et de périkymaties. Les habitudes de brossage et la qualité des dentifrices expliquent les modifications de surface.

Abstract—The study of surface enamel by means of specimens have shown that a considerable number of teeth must be used, and it is necessary to express the results in a statistical way. Under these conditions a technique permitting the reduction of operating time, as well as of the shadowing of the specimens, will have special interest.

The phase-contrast microscope (Nomarski system) permits a direct examination without shadowing the specimens. To interpret the contrasted images obtained, and to know the exact nature of the relief, it is necessary to know the direction of the tangential light. This is easily obtained by comparison.

From the many examinations thus made practicable, it is possible to elucidate certain controversial matters. The experiences indicate that "Perikymaties" are formed by the fusion of several Retzius lines, and explains the qualities of hardness and resistance to surface acids.

The erosions are not recovered by cuticle, and the prime causes of their formation are predominantly mechanical. Human teeth from the Neolithic Age show the presence of cuticle and of "Perikymaties" brushing habits and the quality of dentrifices explain the surface modifications.

Zusammenfassung—Replicastudien der Schmelzoberfläche haben gezeigt, daß sie an einer beträchtlichen Anzahl von Zähnen durchgeführt und statistisch ausgewertet werden müßen. In dieser Hinsicht ist eine Methode von Interesse, die einen Arbeitsgang einspart, andererseits aber ähnlich empfindliche Ergebnisse liefert wie mit der Schattenerzeugung bei Replicapräparaten.

Das Phasenkontrastmikroskop (System Nomarski) erlaubt eine direkte Untersuchung ohne die sonst notwendigen Replicaschatten. Um die so erhaltenen Kontrastbilder interpretieren und die Natur des Reliefs erkennen zu können, muß die Richtung des tangential einfallenden Lichtes bestimmt werden. Dieses ist durch Vergleich leicht zu erreichen. Zahlreiche in dieser Art durchgeführte Untersuchungen haben dazu beigetragen, gewiße widersprechende Punkte aufzuklären. Die Experimente konnten Hinweise dafür liefern, daß die Perikymatien durch Fusion mehrerer Retzius'scher Streifen entstanden sind. Sie erklären so den Härtegrad und die Resistenzstärke der Oberfläche gegenüber Säuren.

Die Erosionen werden vom Schmelzoberhäutchen nicht erneut überdeckt und die Ursache ihrer Entstehung ist im wesentlichen mechanischer Natur.

Menschliche Zähne der neolitischen Epoche zeigen das Vorhandensein des Schmelzoberhäutchens und von Perikymatien.

Die Art des Zähnebürstens und die Qualität der Zahnpasten erklären die Unterschiede in der Beschaffenheit der Schmelzoberflächen.

INTRODUCTION

LES ÉTUDES récentes des caries précoces de l'émail par diverses méthodes ont permis de préciser que la carie débute par une décalcification d'un point de la surface de l'émail. La fine structure de cette surface a été l'objet de nombreux travaux et les moyens d'investigations se sont régulièrement étendus. En 1923, PICKERILL observe, en lumière réfléchie, la surface préalablement imprégnée de graphite. En 1938, EISENBERG observe la surface en fond noir, lumière incidente (dispositif Ultropak de Leitz). WOLF, en 1940, met au point le procédé de répliques pelliculables transparentes, examinées au microscope en éclairage oblique. En 1946, SCOTT et WYCKOFF augmentent le contraste du champ par ombrage des répliques, à l'aide d'un mince dépôt de métal évaporé sous vide et déposé obliquement sur la réplique. Examinées en lumière transmise, ces répliques ombrées donnent des images négatives accusant un relief qui est fonction de la structure locale de la surface observée.

Des indications très importantes, relatives à la surface de la dent, ont été publiées grâce à l'examen de répliques ombrées de milliers de dents. Il s'agit, en effet, de déterminer les caractères de ces surfaces en fonction d'un grand nombre de variantes:

(a) dents avant et après éruption
- jeunes et dents anciennes
- ayant subies une abrasion ou non
- saines dans bouche à carie
- cariées avec partie saines examinées dans la bouche avec nombreuses caries ou peu de caries.

(b) Examen de ces divers types de surface,
avec hygiène—fréquence du brossage, usage de dentifrices abrasifs ou non,
sans hygiène
(c) en fonction de la salive à divers pH
(d) en fonction de divers régimes alimentaires
(e) en fonction de divers perturbations d'ordre général
(f) en fonction de diverses thérapeutiques
(g) érosion dans ces diverses groupes
(h) régions entourant la carie dans ces groupes, etc...

Il est évident que ces études présentent un caractère statistique.

MANNERBERG (1960), qui a publié des résultats fort intéressants sur les modifications de surfaces en fonction des habitudes de brossage, a examiné des milliers de répliques dans diverses catégories. SCOTT et WYCKOFF (1946) ont observé à leur tour, des centaines de dents pour préciser la nature de la surface proche des zones cariées.

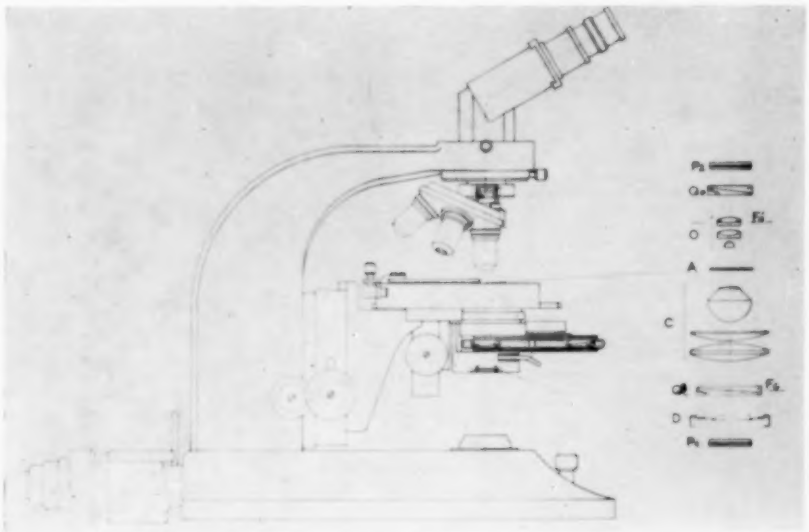
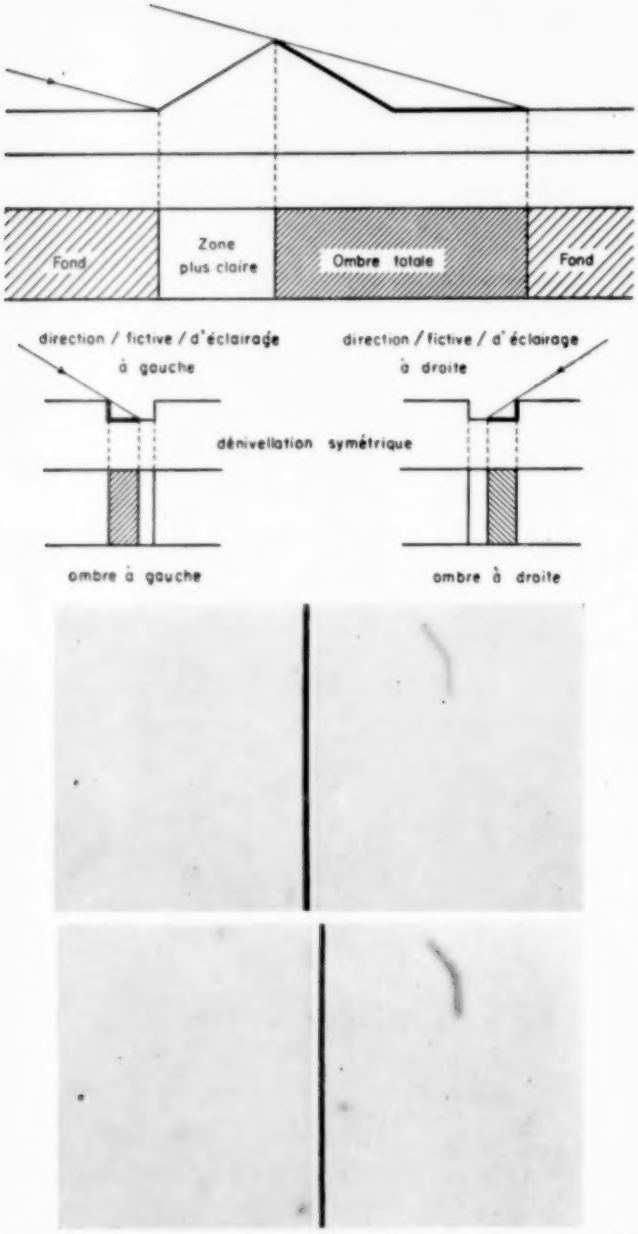


FIG. 1. Schèma du microscope à contraste interférentiel.
*P*1 et *P*2—polariseur analyseur en positions croisées;
D—Diaphragme d'ouverture;
Q_c—bi-prismes en quartz correspondant aux objectifs utilisés placés dans la tourelle sous le condenseur;
Q_o—bi-prisme en quarzt placé dans une monture réglable; latéralement, au-dessus du révolver porte-objectifs;
A—préparation;
O—objectif.



FIGS. 2–4. Principe de l'interprétation des zones claires et foncées suivant le sens du relief et le réglage de l'appareil. La disposition des zones grises et des zones claires permet de déterminer le signe du relief des surfaces examinées. Pour un éclairage oblique dirigé de gauche à droite, la zone claire correspond à une surélévation et les zones foncées à une dépression.

Dans ces conditions, toute méthode permettant l'examen des surfaces en supprimant des temps opératoires aura son importance. La méthode, dite par ombrage, nécessite un dépôt de métal sur la réplique. Cette manipulation est délicate puisque selon la nature, l'angle de dépôt et la qualité du métal, l'image sera différente. Cet ombrage demande des manipulations et des délais supplémentaires et par conséquent les risques d'artefacts pourront être d'autant plus nombreux.

Le microscope à contraste interférentiel (Système NOMARSKI, 1955) permet un examen direct des répliques transparentes préparées par une technique usuelle. Les images obtenues présentent un excellent contraste qui révèle la texture superficielle de l'émail sans qu'il soit nécessaire de les ombrer par métallisation sous vide.

Vol. 6
1961

DESCRIPTION DU MICROSCOPE

Composé d'éléments optiques en quarzt fonctionnant en lumière polarisée placés l'un au dessous du condenseur, l'autre au dessus de l'objectif, il produit une modulation de la lumière diffractée par la préparation et donne naissance au phénomène de contraste par interférences type différentiel.

On a une impression de relief qui révèle les accidents de la structure superficielle de l'objet. Selon le réglage du compensateur Q_0, la direction fictive de l'éclairage peut être dirigée de droite à gauche ou de gauche à droite, ce qui a pour conséquence d'inverser les zones claires et les zones foncées de l'image.

Pour interpréter le sens du relief obtenu lors d'une observation, il faut procéder par comparaison. Dans ce but, on utilise une lame test, constituée par une couche mince déposée sur une lame de verre. Cette lame présente une rainure ou dépression, pratiquée dans l'épaisseur de cette couche. Selon la disposition de l'ombre totale à droite ou à gauche de la zone dénudée, il est facile de connaître le sens fictif de l'éclairage latéral.

OBSERVATIONS

L'examen des surfaces étudiées par la méthode du contraste interférentiel, système NOMARSKI (1955) nous ont permis de confirmer les travaux antérieurs de divers chercheurs, de préciser quelques caractéristiques et de pouvoir présenter des hypothèses qui auront au moins l'intérêt d'orienter des recherches ultérieures.

—Les périkymaties caractérisent les surfaces n'ayant pas subi d'usure mécanique (dents incluses, dents jeunes, dents non soumises à des brossages abrasifs— Figs. 5 et 6).

—Des dents néolithiques adultes examinées ont des surfaces ou subsistent des périkymaties recouvertes de cuticule que nous avons détachées ou détruites mécaniquement (Figs. 10 et 11).

En usant supérficiellement et progressivement les surfaces présentant des périkymaties, nous avons constaté qu'à de faibles profondeurs, les périkymaties disparaissent et ne se retrouvent pas si l'on continue à user progressivement cette surface.

FIG. 5. Troisième molaire inférieure incluse. Compensateur déplacé à gauche (voir Fig. 2), 100 mμ, 14 mm. Microscope Nochet, obj. 10/0,25.

FIG. 6. Troisième molaire inférieur incluse. Compensateur déplacé à droite (voir Fig. 3), 100 mμ, 14 mm. Microscope Nochet, obj. 10/0,25.

Vol. 6
1961

FIG. 7. Extrémités de prismes et substance interprismatique (dent incluse 40). 100 mμ, 50 mm.

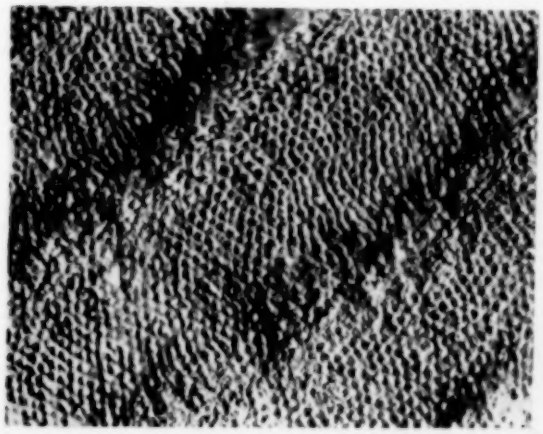


FIG. 8. Extrémités de prismes vues à un fort grossissement avec périkymaties—dent incluse. Microscope Nachet, obj. 25 X, 100 mμ, 28 mm.

Il en résulte que les périkymaties seraient une structure de la surface. Comme l'a montré GUSTAFSON (1959), elles seraient formées par la fusion de plusieurs lignes de Retzius.

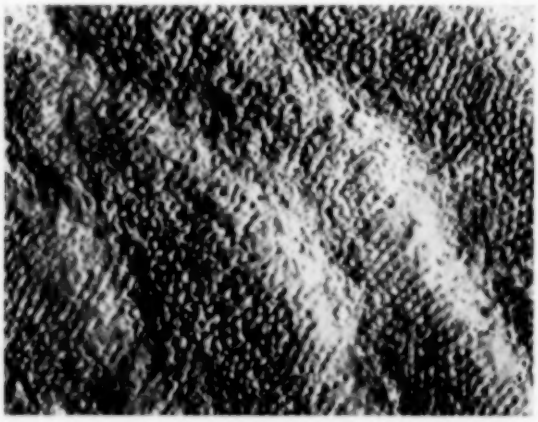


FIG. 9. Périkymaties en voie de disparition. Microscope Nachet, obj. 25 X, 100 mμ, 28 mm.

Les micrographies de la Figs. 5 et 6 montrent une même portion d'émail examinée au contraste interférentiel réglé de façon à ce que le relief soit éclairé de gauche à droite (Fig. 5) ou bien de droite à gauche (Fig. 6). Le profil en forme

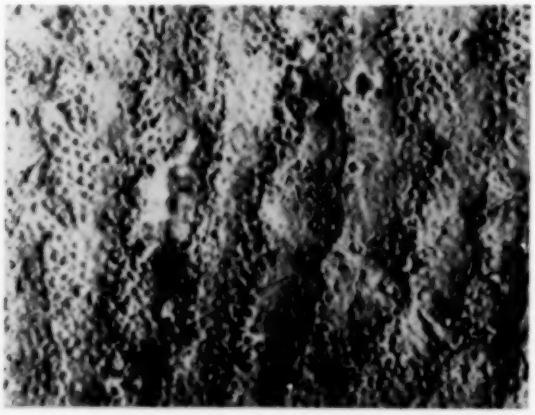


FIG. 10. Surface d'une première molaire inférieure de l'époque néolithique. Les périkymaties et les extrémités de prismes sont apparentes. Obj. 15, ocul. 8.

de vagues n'étant pas régulier, ce double examen en permet une étude plus précise. Cette inversion s'obtient simplement en agissant sur la vis de réglage, alors que dans le cas d'ombrage métallique il faudrait réaliser deux préparations distinctes.

De nombreux faits d'expérience renforcent l'hypothèse du rôle protecteur de la cuticule. Les forces de friction la détruisent plus ou moins et elle se reforme constamment par précipitation de mucine et de sels minéraux. Elle ne disparaît que

FIG. 11. Surface vestibulaire d'une molaire inférieure à face occlusale très abrasée. Les extrémités de prismes ne sont plus visibles. La structure annelée subsiste encore. La strie verticale pourrait résulter du contact d'un aliment dur. Obj. 25, ocul. 8.

lorsque les forces abrasives (pâte dentifrice abrasive) sont trop puissantes pour permettre sa reconstitution progressive. Ceci expliquerait sa présence et la présence de périkymaties sur des dents néolithiques adultes et leur absence sur les lacunes cunéiformes provoquées par les pâtes abrasives.

CONCLUSION

L'examen des répliques au moyen du microscope à contraste interférentiel système Nomarski permet d'observer directement, d'une façon plus simple que par les méthodes usuelles, les états des surfaces.

Nous avons présenté des images des surfaces obtenues à l'aide de ce procédé, dont la précision et la simplicité nous permettront de progresser dans cette étude.

Remerciements—Ce travail a été réalisé à l'Institut d'Optique Théorique et Appliquée (Paris), grâce aux précieux conseils et à l'aide de Monsieur Nomarski auquel nous exprimons notre reconnaissance.

RÉFÉRENCES

BESSIS, M. et NOMARSKI, G. 1957. Les cellules du sang vues au microscope à interférences (système Nomarski). *Rev. d'Hemat.* **12**, 518–528.

EISENBERG,. 1938. A microscopical study of the surface enamel of human teeth. *Anat. Rec.* **71**, 221.

GUSTAFSON, A. G. 1959. A morphologic investigation of certain variation in the structure of enamel. *Odont. Tidskr.* **67**, 361.

Vol. 6
1961

MANNERBERG,. 1960. Appearence of tooth surface. *Odont. Revy* **7**, (Suppl. 6).

NOMARSKI, G. 1955. Microinterferomètre différentiel à ondes polarisées. *J. Phys. Radium.* **16**, 9–13.

NOMARSKI, G. 1951. Du contraste de phase au contraste par interférences. *Rev. d'Hemat.* **12**, 439–442.

NOMARSKI, G. et WEILL, A. T. 1955. Application à la métallographie des méthodes interférentielles à deux ondes polarisées. *Rev. Met.* **52**, 000–000.

PICKERILL, A. 1923. Prévention of Dental Caries. Baillière, Tindall & Cox, London.

SCOTT, A. and WYCKOFF, A. 1946. Shadowed replicas of tooth surfaces. *Publ. Hlth Rep. Wash.* **61**, 697.

WOLF, A. 1940. Plastische Histologie der Zahngewebe. *Dtsch. Zahn-, Mund- u. Kieferheilk.* **7**, 265–284.

Vol.
196

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 149–160, 1961. Pergamon Press Ltd.

NEW INVESTIGATIONS INTO THE CARIOGENIC PROPERTIES OF DENTAL PLAQUES

H. EGGERS-LURA, Dr. Med. Dent.
Algade 28, Holbaek, Denmark

Abstract—The biochemical properties of dental plaques are very different from those of the saliva, a fact which probably explains their cariogenicity. The author emphasizes especially the more concentrated bacterial content of the plaques, the slime and polysaccharide formation due to synthetic effect of certain bacterial plaque strains, the ammonium production and the high buffer capacity, the high pH and the low oxido-reduction potential, the anaerobism, the very poor content of ionized inorganic phosphate, the high content of nucleic acids and ATP which are able to complex the enamel calcium, the lack of amylase and oxidase, the presence of other enzymes such as phosphatase, esterase, galactosidase and beta-D-glucoronidase. Opposite to what happens in saliva, the retarded sugar clearance and the accumulation of sucrose will prevent fermentation and acid formation in the plaque in which the formation of saccharates with complexing properties may accelerate the initial caries attack upon the enamel at neutral pH. The plaque accelerates the caries process, not because acids are formed in it by sugar degradation, but rather because it is unable to form acids to the same degree as saliva.

Zusammenfassung—Die biochemischen Eigenschaften der Zahnplaques sind sehr verschieden von denen des Speichels, eine Tatsache, die wahrscheinlich ihre kariogenen Eigenschaften erklärt. Der Autor hebt besonders den höheren Bakteriengehalt der Plaques hervor, dann den Schleim und die Polysaccharidbildung durch gewisse Bakterienstämme in den Plaques, die Ammoniumbildung und die höhere Pufferkapazität, den höheren pH-Wert und das niedrigere Redoxpotential, die anaeroben Verhältnisse, den sehr niedrigen Gehalt an ionisiertem anorganischem Phosphat, den hohen Gehalt an Nucleinsäure und ATP, die Fähigkeit zur Komplexbindung des Calciums aus dem Zahnschmelz besitzen, den Mangel an Amylase und Oxydase, das Vorhandensein anderer Enzyme wie Phosphatase, Esterase, Galaktosidase und Beta-D-Glukuronidase.

Im Gegensatz zu den Verhältnissen im Speichel vermindert die verzögerte Zuckerclearance und die angehäufte Saccharose die Säurebildung in den Plaques in Verbindung mit der Bildung von Saccharidderivaten Diese haben die Fähigkeit Komplexbindungen einzugehen, und beschleunigen vielleicht den anfänglichen Kariesangriff auf den Zahnschmelz bei neutralem pH. Die Plaques beschleunigen den Kariesprozeß, nicht weil Säuren beim Zuckerabbau gebildet werden, sondern eben weil sie nicht im selben Maße wie Speichel Säure bilden können.

INTRODUCTION

TODAY we may confine the main facts about caries aetiology to the following: (1) Bacterial accumulations such as found in plaques are necessary for the develop-

ment of caries. Lack of bacteria or bacteria occurring in low concentrations—such as found in saliva—are not able to start the initial caries attack (ORLAND, BLAYNEY and HARRISON, 1954); (2) Refined sugars accumulated at certain concentrations are caries provoking. Diluted sugar solutions are not caries provoking (VIPEHOLM REPORT, 1952; EGGERS-LURA, 1954).

As a matter of fact caries never starts directly on surfaces washed by the saliva, caries always starts under the dental plaque. Plaques are found on both sound and carious tooth surfaces. Although no specific qualitative differences either microscopical, bacteriological or biochemical have been established until now between plaques from carious and non-carious teeth, some quantitative differences have been observed and some authors have claimed a greater acid-forming power of the caries suspicious plaque due to its greater content of bacteria and sugar. However this assertion has never been really proved and quite as many investigators came to the opposite result (MILLER, MUNTZ and BRADEL, 1940; ENGLANDER, CARTER and FOSDICK, 1956; TOVERUD and HAGEN, 1957; FROSTELL and RHODIN-BLOMBERG, 1957; EGGERS-LURA 1958a,b; BRAMSTÅNG and NYSTROM, 1961). pH measurements in animals (rats and monkeys) in carious areas have failed to detect a fall below 7 even after carbohydrate food ingestion (OCKERSEE and DOJAGER, 1957; HALDI *et al.*, 1960). The buffer capacity of the plaque seems to be much greater than that of the saliva (STRÅLFORS, 1950) and also the ammonia and alkali forming power is much greater than that of the saliva (FROSTELL, 1960). Actually the initial pH of human caries in plaques has never been measured exactly *in vivo* (SCHATZ and MARTIN, 1955) and it can hardly be much below neutrality because proteolytic bacteria which are constantly found in these sites, do not ordinarily develop in acid milieu. So we are allowed to conclude that the cariogenic property of the plaque may lie, not in its acid forming faculty, but in other factors which have not yet been sufficiently elucidated. It was the aim of the present author to examine such factors by a series of experiments.

MATERIAL AND METHODS

The plaque material was scraped from the teeth 4 hr after the last meal. Before its removal the teeth were washed by a stream of water and isolated from the saliva by cotton rolls. The material (generally amounting to from 0·5 to 2 mg dry wt.) was suspended in distilled water and thoroughly stirred before the experiments. The patients were classified according to the criterion used by the author in previous investigations, including (1) a caries-resistant group (C-R), (2) a questionable resistant group (C-R?) and (3) a caries-active group (C-A). The pH and E_h were measured electrometrically by means of a pH-meter (Radiometer 22) with glass, calomel and platinum electrodes respectively, except that in measurements made *in situ* indicator paper was used.

Experiment No. 1

TABLE 1. pH OF THE PLAQUES *in situ*
(MEASURED COLORIMETRICALLY WITH LYPHAN-INDICATOR-PAPER No. 666)

No.	C-R group	C-R? group	C-A group
1	6·7	6·6	7·1
2	7·1	7·4	7·4
3	6·4	7·2	7·2
4	6·2	6·9	7·2
5	7·2	7·1	6·9
6	6·8	6·7	7·2
7	7·2	7·1	6·9
8	6·5	6·8	7·1
9	6·9	7·0	6·8
10	7·1	6·8	7·4
11	7·0	7·1	6·9
12	6·9	6·8	7·0
M	6·8±0·31	6·9±0·17	7·1±0·12

No statistical significance between the different groups could be found. This con forms with the results of STEPHAN (1938) and MILLER *et al.* (1940). The author's values seem to be a little higher than those of the previous authors, probably due to more careful isolation from the saliva. The pH values of saliva are generally lower than the pH values of the plaques (cf. ammonium production, FROSTELL, 1960).

Experiment No. 2

TABLE 2. THE pH AND THE CORRESPONDING E_h OF PLAQUE SUSPENSIONS
(1–2 mg plaque material (dry wt.) was suspended in 1 ml distilled water. Both pH and E_h measurements were performed electrometrically immediately after the collection)

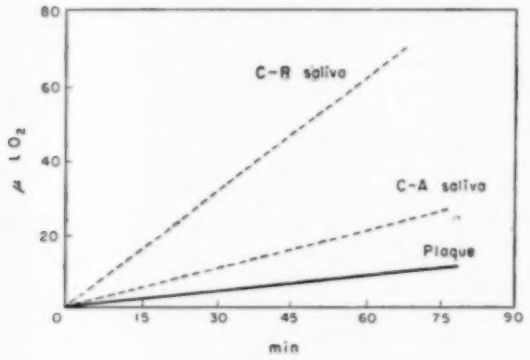
No.	C-R group		C-R? group		C-A group	
	pH	E_h	pH	E_h	pH	E_h
1	7·4	+ 240 mV	6·9	+ 180 mV	7·2	+ 160 mV
2	6·9	+ 177 mV	7·1	+ 190 mV	6·9	+ 175 mV
3	7·2	+ 155 mV	6·9	+ 146 mV	7·0	+ 168 mV
4	6·8	+ 210 mV	6·9	+ 190 mV	7·3	+ 130 mV
5	6·7	+ 210 mV	7·1	+ 140 mV	6·9	+ 140 mV
6	7·2	+ 180 mV	7·0	+ 175 mV	7·1	+ 142 mV
7	6·8	+ 210 mV	6·7	+ 240 mV	7·0	+ 190 mV
8	7·1	+ 212 mV	6·9	+ 190 mV	7·1	+ 130 mV
9	6·9	+ 128 mV	7·0	+ 200 mV	6·9	+ 180 mV
	7·0	+ 191 mV	6·93	+ 183 mV	7·03	+ 157 mV

The lowest E_h values were found in the caries group. Generally the E_h values of the plaque suspensions are lower than the E_h of the corresponding E_h values of the saliva. The E_h of the saliva has formerly been estimated by EISENBRANDT (1945) at +301 mV (mean of 1555 readings), by TURNER and CROWELL (1947) at +309 mV and EGGERS-LURA (1956) from +237 mV to 309 mV (the means of C-R and C-A)

Experiment No. 3

TABLE 3. THE OXYGEN UPTAKE OF THE PLAQUE MICROFLORA

(1 ml plaque suspension in water +0·5 ml phosphate buffer pH 7·1 in Warburg manometer. Control: 1 ml C-R unstimulated saliva in buffer)



The plaque respiration is very poor compared with that of saliva indicating that the plaque microflora must be mainly anaerobic or lacking oxidation activating substances such as cytochrome or hydrogen acceptors which are abundant in normal saliva.

Experiment No. 4

O_2 *solubility of plaques*

The O_2 solubility curves for the saliva have been calculated electrometrically by the author (1955) to 0·19 vol % at 22°C. Using the same method with plaque suspensions of different concentrations the O_2 solubility was found to be much lower in plaques (about 0·05 vol %).

According to STRÅLFORS (1956) the oxygen pressure of plaques was calculated to be zero from a depth of 0·32 mm. The plaques of the teeth very often have a thickness surpassing this measure. So we may assume that anaerobic conditions are prevailing in the dental plaques.

Vol. 6
1961

Experiment No. 5

TABLE 4. THE CONTENT OF INORGANIC, SOLUBLE P IN PLAQUES
(Method: EGGERS-LURA (1947) Material filtered twice)
No acid reagents.

C-R plaques (mg %)	C-A plaques (mg %)
1·2	0·2
1·8	0·9
1·2	1·6
0·8	0·7
1·7	1·6
1·6	0·9
0·9	0·7
1·8	0·3
1·9	1·1
1·8	0·8
M=1·47	M=0·88

The content of inorganic, soluble P in the plaques is especially small in the C-A group. Compared with the content of inorganic, soluble P of the saliva (average 14·9 mg %: EGGERS-LURA, 1947) the average content of plaques only constitutes a tenth of that in saliva.

Experiment No. 6

TABLE 5. THE CONTENT OF ORGANIC, INSOLUBLE P IN PLAQUES
(Material: 0·5 mg plaque (dry wt.); Method: EGGERS-LURA, 1947)

C-R plaques (mg %)	C-A plaques (mg %)
2·2	3·2
2·5	4·3
1·9	2·7
2·1	1·8
2·8	2·6
3·1	2·8
2·9	2·9
3·4	2·2
2·4	3·2
M=2·59	M=2·83

The content of organic, acid insoluble P in plaques is considerably greater than that in the saliva. (Saliva: average 0·6 mg %) possibly due to the more concentrated microbial content of cell-nucleoproteides and the presence of amylopectine (GIBBONS, SOCRANSKY and MACDONALD, 1961).

Experiment No. 7

Adenosinetriphosphate (ATP) content of plaques

By means of the paper-chromatographic method of EGGLESTON and HEMS (1952) and the Boehringer enzymatic test method (TC-J 15979) a series of analyses of the ATP content in plaques were performed (Average of twenty analyses).

C-R plaques	C-A plaques
25–80 gamma/ml	50–420 gamma/ml

The content of ATP in plaques is greater than the content in saliva and all the highest ATP- values were found in C-A persons.

Average content of ATP in saliva

C-R persons: 0–50 gamma/ml (NAUJOKS *et al.*, 1958)

C-A persons: 0–217 gamma/ml

Sugar phosphoric esters in plaques

By means of the paper-chromatographic method of HANES and ISHERWOOD (1949) several phosphoric esters of the type of fructose-1,6-diphosphate, glucose phosphate, mannose phosphate and glycerophosphate could be detected in greater quantities than in the saliva, but the exact identification offered certain difficulties due to the great solubility of these esters.

Experiment No. 8

TABLE 6. THE STARCH FERMENTATION IN PLAQUES
2 mg plaque (wet wt.) in 2 ml 1% starch solution in buffer pH 7·1
Control: 1 ml saliva + 1 ml sol. starch in the same buffer. Incub. 37°C
Average of twenty experiments

Incubation (hr)		Plaque		Saliva	
		Aerobe (mV)	Anaerobe (mV)	Aerobe (mV)	Anaerobe (mV)
0	pH	7·1	7·1	7·1	7·1
	E_h	+210	+190	+310	+304
4	pH	7·1	7·0	6·5	6·1
	E_h	+180	+170	+301	+180
12	pH	7·0	6·9	5·5	4·8
	E_h	+168	+160	+280	+210

The experiment shows that fermentation of starch does not take place in the plaque, especially at high pH, contrary to the saliva where the pH will sink very fast. Amylase is present both in saliva and in plaques, but the plaque bacteria is different from that of saliva in being incapable of fermenting starch directly at pH 7·1.

Vol. 6
1961

Experiment No. 9

TABLE 7. PHOSPHATASE CONTENT OF PLAQUES
Method: phenylphosphate method (EGGERS-LURA; 1949). In Sharer units. (Mean of twenty-two experiments.)

Plaque		Saliva	
pH 5·5	pH 9·4	pH 5·5	pH 9·4
6·2	3·1	2·8	2·1

The content of phosphatase in plaques is more concentrated than in saliva.

*Esterase, Galactosidase and Beta-*D*-Glucoronidase*

LISANTI (1961) found bacterial content of esteresa, galactosidase and beta-D-glucoronidase in caries areas. These experiments could be confirmed by using dental plaques from carious persons as material. The action is mainly synthetizing.

Oxidase in Plaques

The presence of cytochromoxidase in saliva has been demonstrated by the author in an earlier experiment (EGGERS-LURA, 1956). Using plaque as material the experiment was negative. Using alpha-naphtol + paraphenylendiamine as reagent (EGGERS-LURA, 1949) the localization of oxidase in plaque material also appeared to be negative.

Synthetizing Enzymes

The presence of bacteria which are able to synthesize mucous polysaccharides from sucrose has been demonstrated by SNYDER *et al.* (1955) Polysaccharide synthesis of the glycogen-amylopectine type for the purpose of storage by the plaque bacteria was demonstrated by GIBBONS *et al.* (1961). Such synthesis was confirmed by control experiments by the author. On the other hand direct fermentation of these polysaccharides with acid formation by means of the plaque bacteria does not seem to take place *in situ* (cf. Expt. No. 8) and at alkaline pH.

Sugar Clearance

By means of a new enzyme test method (glucose-oxidase-Tess-tape-method) (EGGERS-LURA, 1961) the author has been able to measure the sugar content of saliva and plaques directly in the mouth within limits of 0·1-2 per cent of glucose. The method is quite specific and not disturbed by other reducing substances in the mouth. By comparison with other methods (such as the Somogyi method used in the Vipeholm report, 1952) it appeared to be sufficiently exact for the determination of the relative values in sugar clearance experiments. In the following experiments the end-point of the sugar clearance (disappearance of colour of the Tes-

tape) was chosen at 0·1 per cent, because this level is optimum for the further oxidation and elimination of the sugar by bacterial respiration. On the other hand concentrations of more than 2 per cent will inhibit or stop further oxidation (EGGERS-LURA, 1954).

Experiment No. 10

Time for 10% glucose or 1% starch solution to reach the level of 0·1%
(average of 104 experiments)

I

Saliva	*Plaques*
2–5 min (average 3·2)	4–12 min (average 6·7)

The sugar clearance of glucose and starch is rather fast both in saliva and in plaques.

II

Time for a piece of candy (sucrose initial concentration about 10%) to reach the level of 0·1%.

(average of 128 experiments)

Saliva	*Plaques*
6–24 min (average 14·3)	18–49 min (average 30·2)

The sugar clearance of sucrose is slower than the sugar clearance of glucose and starch.

III

Time for a piece of candy to reach the level of 0·1% following the intake on four occasions at 15 min intervals.

(average of 110 experiments)

Saliva	*Plaques*
20–45 min (average 32·5)	30–68 min (average 47·4)

Repeated intakes of sugar seem to inhibit the sugar clearance both in plaque and in saliva. An accumulation of sugar seems to take place under these circumstances.

IV

The same method of experiment (repeated intakes of sugar) as in III but using C-R plaques and C-A plaque material.

(average of 86 experiments)

C-R plaque	*C-A plaque*
24–47 min (average 38·4)	35–74 min (average 51·2)

The sugar clearance of plaques after repeated intakes of sugar is slowest in the C-A plaque material.

Content and Change of Sialic Acid

Both in saliva and in plaques the content of sialic acid can be demonstrated (EGGERS-LURA, 1960). The content of sialic acid is raised in saliva of caries active persons. In plaques a synthesizing effect can be observed by addition of pure sialic acid and sucrose to the plaques. The carbohydrate moiety seems to be stored as polysaccharides (cf. GIBBONS *et al.*, 1961).

Experiment No. 11

TABLE 8. ATP-FORMATION IN PLAQUE–SUCROSE MIXTURES
(Enzyme-test method by Boehringer TC-J 15979)

Material	Incubation (hr)	ATP (gamma/ml)	pH	Soluble Ca (mg %)	Soluble P (mg %)
Plaque (1 mg dry wt.) +1 ml 5% sucrose	0	37–268	7·1	3	1·2
Plaque + 1 ml 5% sucrose +50 mg $Ca_3(PO_4)_2$	2–3	449–617	7·0	17	8

(average of twelve experiments)

Incubation with plaque material + sucrose + insoluble tricalciumphosphate-increases the ATP content and the level of soluble Ca and P. This fact seems to support the caries theory of the author (EGGERS-LURA, 1961a, b).

DISCUSSION

The biochemical properties of dental plaques are different from those of saliva, a fact which probably explains their cariogenicity. In a series of experiments the author could demonstrate such differences summarized in Table A and Table B. The sugar clearance of plaques is slower than of saliva and the accumulation of sucrose more frequent. Concentrations above 2% are very rare in saliva over longer periods, but not rare in the plaques. Since the optimum concentration for bacterial oxidation of sucrose in the oral cavity is about 0·1%, the breakdown of sucrose must be inhibited in the plaques, and due to the anaerobic conditions in these sites it will take place by fermentation and not by oxidation, or not at all. Pure saccharates or intermediary products such as lactate, pyruvate, ATP, malate, phosphoglycerate, etc., will accumulate and can be demonstrated in biochemical experiments (EGGERS-LURA, 1958). Blackwell, Mc Millan and Fosdick (1961) have demonstrated that sugar may etch the enamel very quickly at alkaline pH. The author's experiment No. 11 shows that incubation with sugar and insoluble tricalcium phosphate will result in an accumulation of ATP and in an

increase of soluble Ca and P at neutral pH. It has further been demonstrated by the author (1958, 1959) that enamel can be attacked very rapidly at pH 7·2 by nucleates, condensed phosphates, saccharates and by parathyroid hormone as well as by ethylenediamine tetraacetic acid (EDTA). The histological appearances produced by such experiments are very similar to the histological appearances seen in early clinical caries.

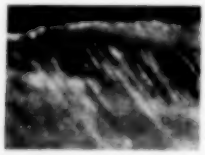


FIG. 1. Clinical caries.

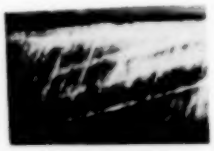


FIG. 2. Chelated enamel.

Gram-positive rods, recently found in increased quantities in carious areas by BRAMSTÅNG and NYSTRØM (1961) do not form acids, but condensed phosphates which are able, like ATP, to complex and dissolve enamel at neutral pH. They also form ammonia from urea (cf. FROSTELL, 1960). The presence of phosphataes in the initial caries lesion has been demonstrated by EGGERS-LURA and ZANDER (1947). Finally it has been shown that fluorine inhibits chelation (SCHATZ and MARTIN, 1960; SOMOGYI, 1957) and complex formation.

TABLE A. SUMMARY OF THE BIOCHEMICAL PROPERTIES OF SALIVA AND PLAQUES

	Saliva	Plaques
Bacterial content	Dilute	Concentrated
Main strains	Lactobacilli	Enterococci
	Streptococci	Streptococci
	Staphylococci	Corynebacterium (gr+)
	Neisseria	Leptotrix
Average pH	6·9	7·1
Average E_h (mV)	+309	+160
Ammonium formation	1	100–400
O_2 uptake (mm^3/hr)	65	0
O_2 solubility (vol %)	0·19	0·05
Buffer capacity	1	8–10
Milieu	Aerobic	Anaerobic
Oxidase activity	Positive	Negative
Starch fermentation	Positive	Negative
Phosphatase activity	Small	Strong
Slime formation	Negative	Positive
Sialic acid (mg %)	8–19	Unknown compounds

TABLE B

	C-R Plaques	C-A Plaques
Inorganic soluble P (mg %)	1·47	0·88
Organic, insoluble P (mg %)	2·59	2·83
Phosphoric esters	Low content	High content
ATP (gamma/ml)	25–80	50–420
Phosphatase (acid) (Sharer units)	5·3	7·4
Sugar clearance (min)	24–47	35–74
Gram positive rods	Limited	Abundant

There is, therefore, considerable evidence that in future caries research will not be based on the formation of acids, that MILLERS' theory will lose much of its significance and that the complex forming enzymatic theory of EGGERS-LURA will gain ground in the coming years (cf. EGGERS-LURA, 1961a, b).

REFERENCES

AHRENS, G. and RANKE, R. 1961. Phosphoric esters in saliva. *Dtsch. Zahnärztl. Z.* **16**, 196.

ÅSLANDER, A. 1960. *Dental Caries*. Institute of Technology. Div. of Agriculture, Report No. 2. Stockholm.

BLACKWELL, R., MCMILLAN, L. and FOSDICK, L. 1961. Sucrose etching of enamel. *J. Dent. Res.* **40**, 16.

BRAMSTÅNG, S. and NYSTRØM, S. 1961. Caries and gram-positive rods. *Odont. Tidsskr.* **69**, 164

EGGERS-LURA, H. 1947. Salivary phosphates. *J. dent. Res.* **26**, 203.

EGGERS-LURA, H. 1949. *Die Enzyme des Speichels*. Hanser Verlag, München.

EGGERS-LURA, H. 1954. Abbau der Kohlehydrate im Speichel. *Dtsch. Zahn-, Mund- u. Kieferheilk.* **21**, 97.

EGGERS-LURA, H. 1955. The E_h of saliva .*Odont. Tidsskr.* **63**, 413.

EGGERS-LURA, H. 1955. Electrochemical measurement of oxygen. *Scand. J. Lab. Invest.* **7**, 181.

EGGERS-LURA, H. 1956. Sauerstoffaufnahme des Speichels. *Dtsch. Zahn-, Mund- u. Kieferheilk.* **24**, 177.

EGGERS-LURA, H. 1957. Nicht-saure Wirkstoffe in Karies. *Dtsch. Zahnärztl. Z.* **12**, 806.

EGGERS-LURA, H. 1958a. Säurebildung in Plaques. *Dtsch. Zahn-, Mund- u. Kieferheilk.* **28**, 447.

EGGERS-LURA, H. 1958b. Experimentelle Schmelzkaries. *Dtsch. Zahnärztl. Z.* **13**, 353.

EGGERS-LURA, H. 1959. ORCA- Report IV. In print.

EGGERS-LURA, H. 1958. Mikrobiologische Mechanismen in Karies. *Dtsch. Zahnärztl. Z.* **13**, 878.

EGGERS-LURA, H. 1960. Sialinsäure im Speichel. *Dtsch. Zahnärtlz. Z.* **15**, 963.

EGGERS-LURA, H. 1961a. Neue Zuckerbestimmung. *Dtsch. Zahnärztl. Z.* **16**, 646.

EGGERS-LURA, H. 1961b. Resorption and caries. *N. Y. St. dent. J.* **27**, 75.

EGGERS-LURA, H. and ZANDER, H. 1947. Phosphataes in caries. *J. dent. Res.* **26**, 473.

EGGLESTON, L. and HEMS, R. 1952. Paperchromotography. *Biochem. J.* **52**, 156.

EISENBRANDT, E. 1945. E_h of saliva. *J. dent. Res.* **24**, 247.

ENGLANDER, H., CARTER, J. and FOSDICK, L. 1956. Plaque acid. *J. dent. Res.* **35**, 792.

FROSTELL, G. 1960. Ammonia production of plaques. *Acta odont. scand.* **18**, 29.

FROSTELL, G. and RHODIN-BLOMBERG, 1957. *Svensk Tandl. Tidsskr.* **7**, 407.

GALE, E. F. 1951. *Chemical Activities of Bacteria*, p. 119.

GIBBONS, R., SOCRANSKY, S. and MACDONALD, J. 1961. Polysaccharide storage of bacteria. Int. Ass. Dent. Res. 39th meeting report. Abstr. 74.

HALDI, J., WYNN, W., LAW, M. and BENTLEY, K. 1960. pH on the teeth of albino rats under various conditions conducive to dental caries. *Arch. Oral Biol.* **2**, 46.

HANES, C. and ISHERWOOD, F. 1949. Paperchromatography. *Nature, Lond.* **164**, 1107.

LISANTI, V. F. 1961. Enzymes in caries. Int. Ass. Dent Res. 39th meeting report. Abstr. 124.

MANLY, R. S. 1961. Sugar and salivary sediment. *J. dent. Res.* **40**, 379.

MILLER, B., MUNTZ, J. and BRADEL, S. 1940. Acid in plaques. *J. dent. Res.* **19**, 473.

NAUJOKS, R., BRAMSTED, F., KRÖNCKE, A. and MARG, H. 1958. Fluorine and energy rich. phosphate. ORCA report V, p. 82.

OCHERSEE, T. and DEJAGER, L. 1957. Caries in monkeys. *Brit. dent. J.* **102**, 93.

ORLAND, F., BLAYNEY, J. and HARRISON, R. 1954. Germ-free caries. *J. dent. Res.* **33**, 147.

SCHATZ, A. and MARTIN, J. 1955. Lactobacillus as anti-caries factor. *N. Y. St. dent. J.* **21**, 367.

SCHATZ, A. and MARTIN, J. 1960. Proteolysis chelation. *N. Y. St. dent. J.* **30**, 124.

STEPHAN, M. 1938. pH of plaques. *J. dent. Res.* **17**, 251.

STRÅLFORS, A. 1950. Bacterial Chemistry of dental plaques. Diss., Stockholm.

STRÅLFORS, A. 1956. Respiration of bacteria. *Svensk. tandläk. Tidsskr.* **49**, 387.

SOMOGYI, J. C. 1957. *Odont. Revy.* (IV. ORCA report) **8**, 207.

TOVERUD, G. and HAGEN, R. 1957. Acid formation in plaques. *Norske Tandlaegeforen. Tid.* **67**, 151.

VIPEHOLM REPORT. 1952. *Svensk. tandläk Tidsskr.* **45**, Suppl.

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 161–173, 1961. Pergamon Press Ltd.

HISTOLOGISCHE STUDIEN AN NAGEZÄHNEN DES SYRISCHEN GOLDHAMSTERS

H. TAATZ

Gr. Steinstrasse 19, Halle/Saale, Deutschland

Zusammenfassung—Ein wertvolles Testobjekt zur Darstellung von Mineralisationsstörungen, die durch Mangelkost oder andere Stoffwechselstörungen hervorgerufen werden können, stellen in der experimentellen Kariesforschung, wie PROELL, SPRETER v. KREUDENSTEIN, KEIL, TAATZ, u.a., beobachten konnten, die Nagezähne syrischer Goldhamster oder die der Ratten dar. Die Ausführungen geben auf Grund der großen Bedeutung der Nagezähne für die weitere Forschung auf diesem so wichtigen Gebiet einen Überblick über die Entwicklung und den Aufbau der Nagezähne, und zwar in dem Umfang, wie es für das Verständnis der entwicklungsbedingten Zusammenhänge erforderlich ist. Die Untersuchungen beschränken sich nur auf die Nagezähne des syrischen Goldhamsters, der, wie aus der Literatur hervorgeht, immer mehr Verwendung in der experimentellen Kariesforschung findet.

Abstract—Experimental research on the subject of caries (PROELL, SPRETER VON KREUDENSTEIN, KEIL, TAATZ, and others) has shown that the canines of Syrian golden hamsters or rats are valuable test subjects for the presentation of mineralization disturbances which may materialize through food deficiencies and other digestive troubles. The canine teeth are extremely important for further research on this essential subject; therefore the report presents a survey of the development and structure of canines to such an extent as is needed for an understanding of the aspects conditioned by trends of development. The tests are limited to the canine teeth of Syrian golden hamsters which are increasingly used in experimental caries research as is shown in literature on the subject.

EIN wertvolles Testobjekt zur Darstellung von Mineralisationsstörungen, die durch Mangelkost oder andere Stoffwechselstörungen hervorgerufen werden können, stellen in der experimentellen Kariesforschung die Nagezähne syrischer Goldhamster oder die der Ratten dar. Die folgenden Ausführungen sollen auf Grund der großen Bedeutung der Nagezähne für die weitere Forschung auf diesem so wichtigen Gebiet einen Überblick über die Entwicklung und den Aufbau der Nagezähne geben, und zwar in dem Umfang, wie es für das Verständnis der entwicklungsbedingten Zusammenhänge erforderlich ist. Die Untersuchungen beschränken sich nur auf die Nagezähne des syrischen Goldhamsters, der, wie aus der Literatur hervorgeht, immer mehr Verwendung in der experimentellen Kariesforschung findet.

Gegenüber den Molaren der Hamster, die in gewissem Grade in histogenetischer und anatomischer Hinsicht mit den menschlichen Zähnen verglichen werden können, zeigen die Nagezähne Besonderheiten, die einen Vergleich nur in ganz beschränktem Maße gestatten. Unter die abnormen Besonderheiten fallen die em-

bryonale Keimanlage, durch die ein permanentes Wachstum möglich ist, die Wurzellosigkeit, das einseitige Vorhandensein der Schmelzdecke auf der labialen Fläche und die dadurch bedingte andersartige Strukturierung des Schmelzes.

Interessant ist, daß wir beim Studium von Nagezahnserienschnitten, die quer von ventral nach dorsal gelegt wurden, die gesamten Phasen der Zahnentwicklung in flächenhafter Ansicht beobachten konnten. Beginnend von der embryonalen Keimanlage über die verschiedenen knospen-, kappen- und glockenförmigen Stadien zur Differenzierung der mesenchymalen und epithelialen Anteile bis zur endgültigen Ausbildung der Zähne. Dagegen können bei Hamstermolaren und besonders bei menschlichen Zähnen gleiche Beobachtungen entsprechend der Entwicklung nur über größere Zeitabstände hin durchgeführt werden.

Vol. 196

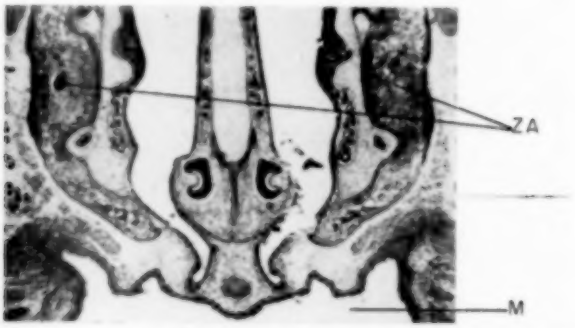


ABB. 1. Cricetus auratus, Übersicht, ZA Nagezahnkeimanlage, M Mundhöhle, 45 : 1, H E-Färbung.

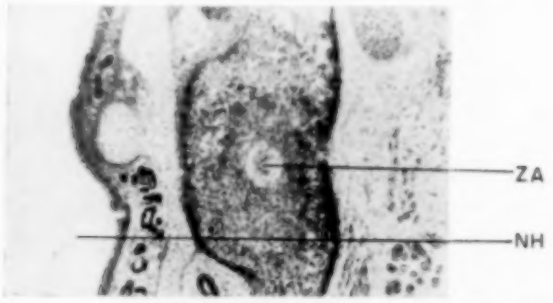


ABB. 2. Cricetus auratus, Vergrößerung der primären Zahnanlage, ZA Zahnkeimanlage, NH Nasenhöhle, 150 : 1, HE-Färbung.

Topographisch entstehen die äußersten im Basalende liegenden Keimanlagen höhenmäßig ungefähr in der Mitte beiderseitig der Nasenhöhlen (Abb. 1). Sie bestehen aus Plattenepithelzellen, die umgeben vom Mesenchym locker eingebettet sind. Zwischen den Plattenepithelzellen befindet sich eine embryonale Grundsubstanz, die histologisch den Charakter einer Interzellularsubstanz hat (Abb. 2). Auf

Vol. 6
1961

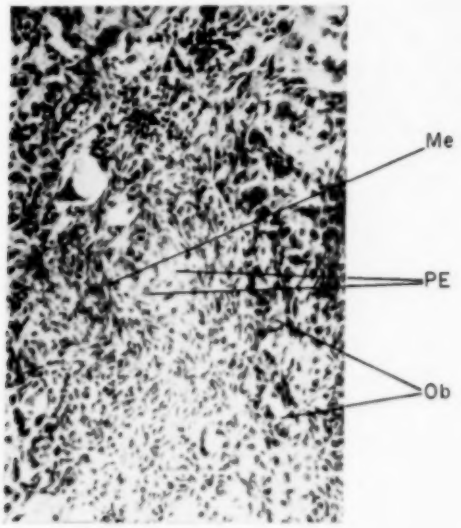


ABB. 3. Cricetus auratus, entsprechende Vergrößerung aus Abb. 2, PE Pflasterepithelzellen, Ob Osteoblasten, Me mesenchymales Gewebe, 300 : 1, HE-Färbung.

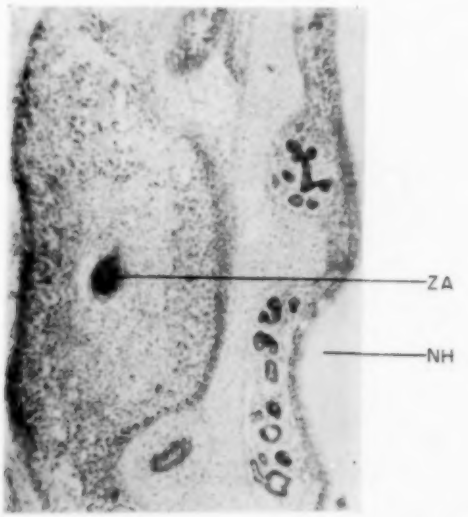


ABB. 4. Cricetus auratus, knospenförmiges Stadium, ZA Zahnkeimanlage, NH Nasenhöhle, 150 : 1, HE-Färbung.

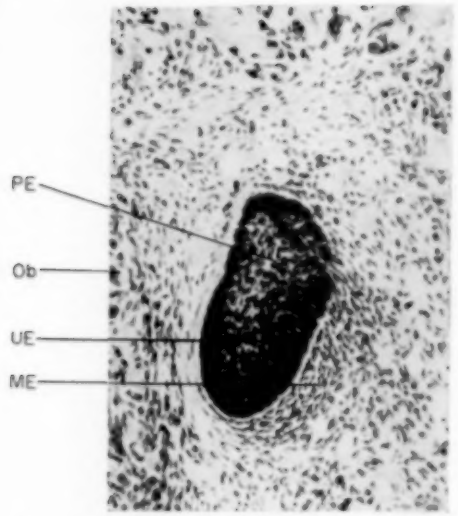


ABB. 5. Cricetus auratus, knospenförmiges Stadium, PE Pflasterepithelzellen, Ob Osteoblasten, UE undifferenzierte Zellen, Me mesenchymale Zellen, 300 : 1, HE-Färbung.

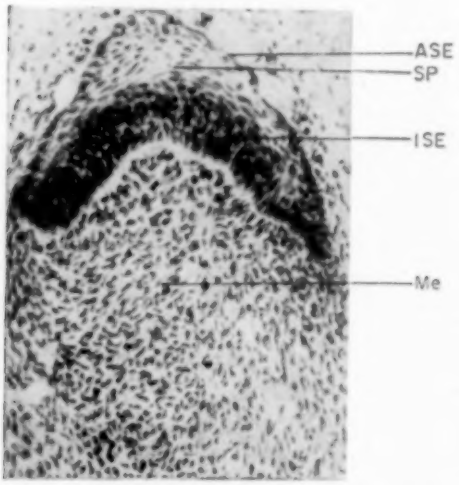


ABB. 6. Cricetus auratus, kappenförmiges Stadium, ASE äußeres Schmelzepithel, SP Schmelzpulpa, ISE inneres Schmelzepithel, Me mesenchymales Gewebe, 300 : 1, HE-Färbung.

Vol. 196

Abb. 3 können wir die Epithelzellen, ihre morphologische Struktur und ihre direkte mesenchymale Umgebung deutlich erkennen. Zur Nasenhöhle hin und lateral sind Osteoblasten festzustellen, welche die Bildung der knöchernen Umgebung übernehmen. Entwicklungsmäßig findet durch Mitose eine Vermehrung und somit Ausbreitung der Zellen und durch den Wachstumsreiz eine Formgebung der primären Zahnanlage statt.

Im knospenförmigen Stadium (Abb. 4) ist im Zentrum der Knospe eine Anhäufung von Plattenepithelzellen vorhanden, während peripher schon, wie die Abb. 5 zeigt, eine Orientierung der Epithelzellen vorliegt. Außerhalb der Knospe ist ungefähr in Diagonallage nach medial eine Verdichtung der mesenchymalen Zellen festzustellen. Die Diagonallage der Nagezahnkeime in diesem Stadium stellt einen Befund dar, den wir in ähnlicher Form von den Hamstermolaren und menschlichen Zähnen her kennen. Auch hier findet im Laufe der weiteren Entwicklung, d.h. wenn man den Zahn weiter nach ventral verfolgt, eine Aufrichtung bzw. Drehung des jeweiligen Zahnanteiles statt. Die am extrahierten Nagezahn angedeutete spiralenförmige Drehung ist entwicklungsmäßig auf die Diagonallage des embryonalen Anteiles des Zahnes zurückzuführen.

Vol. 6
1961

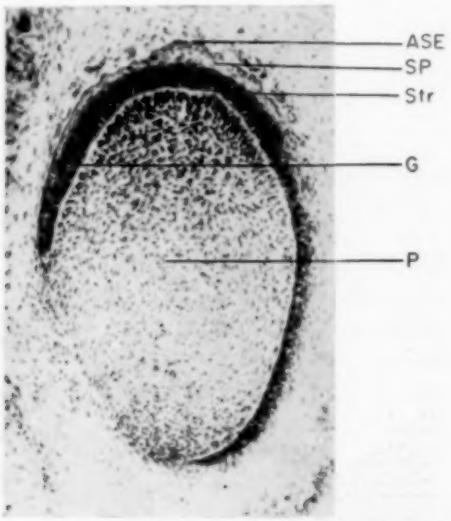


ABB. 7. Cricetus auratus, glockenförmiges Stadium, ASE äußeres Schmelzepithel, Str Stratum intermedium, SP Schmelzpulpa, G Ganoblasten, P Papille, 210 : 1, HE-Färbung.

Die nächste Phase der Entwicklung (Abb. 6) läßt dann, bedingt durch die kappenförmige Ausbildung, das äußere Schmelzepithel, die Schmelzpulpa, das innere Schmelzepithel und die spätere Zahnpapille erkennen. Das äußere Schmelzepithel und die Schmelzpulpa liegen im glockenförmigen Stadium (Abb. 7), im Gegensatz

zu den Molaren im gleichen Entwicklungszustand, dem inneren Schmelzepithel sehr eng an. Direkt über dem inneren Schmelzepithel liegt das Stratum intermedium mit seinen flachen Epithelzellen. Die Schmelzpulpa, die zwischen dem Stratum intermedium und dem äußeren Schmelzepithel liegt, besteht aus sternförmigen Zellen mit runden bis ovalen Kernen, deren Ausläufer netzförmig miteinander verbunden

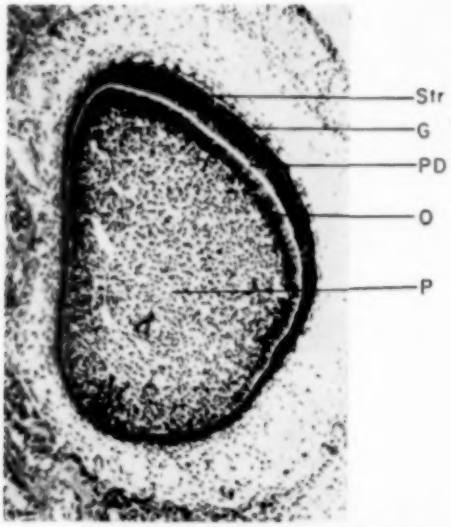


ABB. 8. Cricetus auratus, Str Stratum intermedium, G Ganoblasten, PD Prädentin, O Odontoblasten, P Zahnpapille, (embryonale Pulpa), 150 : 1, HE-Färbung.

sind. Die inneren Epithelzellen sind die Schmelzbildner oder Ganoblasten, die gegenüber den äußeren Epithelzellen Differenzierungen durchmachen (Abb. 8).Die Kerne der Ganoblasten liegen bis auf ganz seltene Ausnahmen stets schmelzpulpawärts. Gegenüber den Ganoblasten und zwar pulpawärts stehen die Odontoblasten. Die Kerne der Odontoblasten sind pulpawärts gelegen. Die Stellung der Gano- und Odontoblasten ist palisadenförmig (Abb. 9).

Der Hohlraum der Glocke wird von einem verdichteten embryonalen Bindegewebe ausgefüllt, der Zahnpapille, der Anlage der späteren Nagezahnpulpa. Ein epithelialer Verbindungsstrang, wie wir ihn von den Molaren her kennen und der eine Verbindung zur Mundhöhle hat, ist beim Nagezahn nicht vorhanden.

Die Nagezahnpulpa dringt mit unverengter Breite in den Zahnkörper ein und läuft zylinderförmig allmählich zur Zahnschneide hin aus. Man kann sagen, daß das Pulpenkavum, bzw. die Pulpa im Quer- und Längsschnitt der äußeren Form des Nagezahnes entsprechen. Die Pulpa besteht aus Mesenchymderivaten und zeigt in ihrem Aufbau keine wesentlichen Unterschiede zur Molarenpulpa. Lediglich finden wir, wie aus der Abbildung 10 hervorgeht, ein größeres Gefäßnetz vor, was

Vol. 6
1961

sicher auf die ständigen Aufbauvorgänge der Zahnhartsubstanzen zurückzuführen ist. Die Stoffwechselvorgänge sind vermutlich physiologisch umfassender und dürften daher eine ausgeprägtere Vaskularisierung verlangen. Die Blutgefäße treten

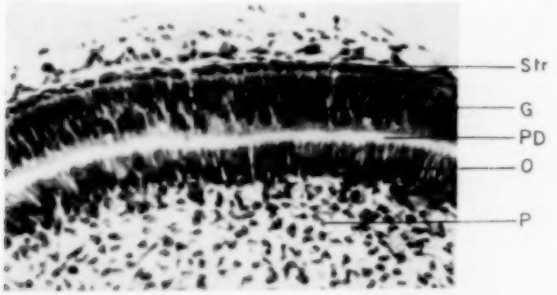


ABB. 9. Cricetus auratus, entsprechende Vergrößerung aus Abb. 8, Str Stratum intermedium, G Ganoblasten, PD Prädentin, O Odontoblasten, P Zahnpapille, 630 : 1, HE-Färbung.

gemeinsam mit den Nerven in ziemlich großer Anzahl am Foramen apikale in das Pulpagewebe ein und teilen sich sehr bald in feinere Zweige auf. Das Kapillar- u. Nervennetz reicht auch bei dem Nagezahn bis in die Odontoblastenschicht.

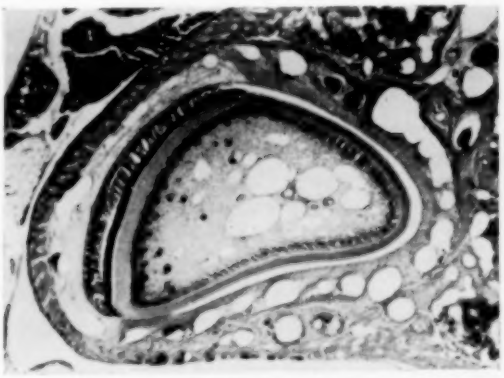


ABB. 10. Cricetus auratus, Nagezahn im Querschnitt, Pulpagefäße, 210 : 1, HE-Färbung.

Die Mineralisation des Dentins, also die Einlagerung von Erdsalzen ist ähnlich wie bei den Hamstermolaren und menschlichen Zähnen. Die einzelnen Schichten entsprechen der äußeren Form der Nagezahnpulpa. Aus dem Gesamtaufbau der Entwicklung ergibt sich, daß sich an der Dentinpulpagrenze stets die jüngsten und an der basalen Schmelzfläche, bzw. auf der palatinalen oder lingualen Außenfläche die älteren Dentinschichten befinden.

Im Gegensatz zu dem Radialschliff (Abb. 11), wo man die Anbaulinien im parallelen Verlauf beobachten kann, liegen sie im Querschliff (Abb. 12) konzentrisch angeordnet. Die dadurch entstehende Streifung bezeichnen wir als Owensche Kon-

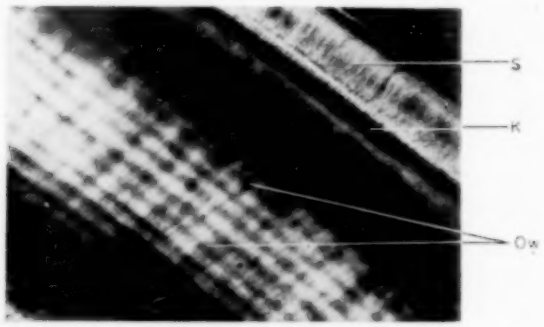


ABB. 11. Cricetus auratus, Radialschliff durch einen Nagezahn, S Schmelz, K v. Korffsches Dentin, Ow Owensche Linien, 150 : 1, Pol. +.

tur- oder Anbaulinien. Interessant ist, wie meine Untersuchungen zeigten, daß die Owenschen Linien in Vielzahl und ausgeprägtem Maße nur bei Tieren auftraten, wo Störungen in der Mineralisation vorlagen. Bei gleichmäßiger Mineralisation konnten dagegen keine Owenschen Linien beobachtet werden.

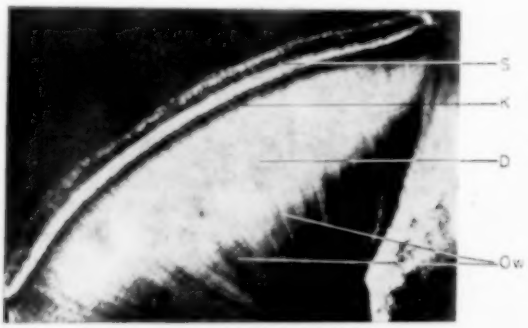


ABB. 12. Cricetus auratus, Querschliff durch einen Nagezahn, S Schmelz, K v. Korffsches Dentin, D Dentin, Ow Owensche Linien, 105 : 1, Pol. +.

Die Zahnbeinkanälchen verlaufen auf der labialen Seite des Zahnes schräg nach inzisal zum Schmelz hin (Abb. 13). Der Verlauf ist fast geradlinig. Lediglich kurz unterhalb des Schmelzes findet eine Abweichung statt. Wir bezeichnen diese Schicht allgemein als v. Korffsches Dentin. Im Radialschliff ist die Schnittrichtung der Kanälchen in der Mehrzahl der Fälle nur im Labialbereich gleichmäßig. Während sie

in diesem Anteil stets längs getroffen sind, finden wir in dem lingualen bzw. palatinalen Bereich häufig quer getroffene Dentinkanälchen, die dadurch dem unteren Teil des Zahnes ein völlig anderes strukturelles Aussehen geben.

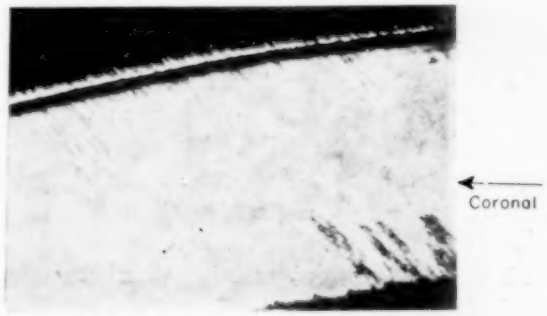


ABB. 13. Cricetus auratus, Radialschliff durch einen Nagezahn, Verlauf der Dentinkanälchen, 150 : 1, Pol. +.

Den bereits bestehenden unterschiedlichen Angaben von KEIL und SPRETER v. KREUDENSTEIN einerseits und PROELL andererseits über den Verlauf der Fibrillen

ABB. 14. Schematische Darstellung des Verlaufes der kollagenen Fibrillen und der Apatitkristallite im Ober- und Unterkiefer. Die kurzen Striche stellen die Kristallite und die langen Striche die Fibrillen dar.

und der Apatitkriställchen bezüglich ihrer Hauptachsenrichtung kann ich mich nur zum Teil anschließen, da meine Untersuchungen—wenn auch geringe—so jedoch sehr interessante Abweichungen gezeigt haben. Die von KEIL angegebene und von

SPRETER v. KREUDENSTEIN bestätigte Verlaufsrichtung, die KEIL in einer Arbeit in Form einer schematischen Darstellung reproduziert hat, trifft nach meinen Untersuchungen nur für den unteren Nagezahn zu. Bei den Oberkiefernagezähnen dagegen tritt eine Abweichung kurz unterhalb der basalen Schmelzschicht auf, wie aus der schematischen Darstellung (Abb. 14) ersichtlich ist.

Auf Grund des fast kreisförmigen Verlaufes der Oberkiefernagezähne ist meines Erachtens diese Anordnung, statisch gesehen, auch einleuchtend; denn die Hauptbelastung der oberen Zähne liegt verständlicherweise auf der labialen Seite, nicht in der Achsenrichtung der Zähne, sondern vielmehr in der gezeigten Richtung. PROELLS Angaben stimmen zwar für die oberen Nagezähne annähernd, aber nicht für die unteren. Den Charakter der Gesamtdoppelbrechung des Dentins habe ich bei meinen Untersuchungen berücksichtigt. Wie schon aus früheren Arbeiten von v. EBNER und W. J. SCHMIDT hervorgeht, wird dieser nicht von den anorganischen kristallinen Bestandteilen, sondern von den kollagenen Fibrillen der organischen Dentingrundsubstanz bestimmt, die auf Grund ihrer stark positiv doppelbrechenden Eigenschaften die negative Eigendoppelbrechung der Kristallite überlagern. Deshalb habe ich die Schliffe entkollagenisiert und anschließend mit verschiedenen Flüssigkeiten, die annähernd die gleiche Brechzahl wie das Mineralgerüst haben, imbibiert. Den Verlauf der Fibrillen und Kristallite konnte ich bei meinen sämtlichen vorliegenden Nagezähnen beobachten.

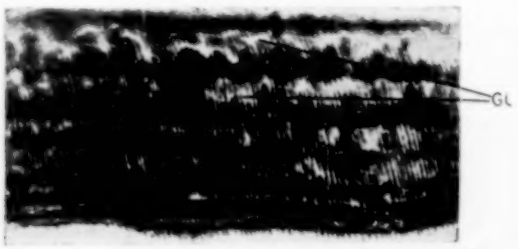


ABB. 15. Cricetus auratus, Radialschnitt durch einen Nagezahn, globuläre Verkalkung des Dentins, Gl Globuli, 300 : 1, HE-Färbung.

Die primäre Verkalkung erfolgt auch bei den Nagezähnen globulär, eine Beobachtung, die bereits PROELL und SPRETER v. KREUDENSTEIN bei ihren Untersuchungen an Rattenzähnen gemacht haben und die ich hiermit auch an Goldhamsterzähnen bestätigen kann (Abb. 15). Im weiteren Verlauf der Entwicklung findet dann—wenn keine Störungen auftreten—eine Einlagerung von Erdsalzen in die Zwischenbezirke der Globuli statt, so daß wir in der Endphase der Entwicklung das homogene Bild der Verkalkung vorfinden. Das globulär mineralisierte Dentin kann somit als Übergangsphase zwischen dem normalen und dem völlig mineralfreien Dentin angesehen werden.

Den Schluß meiner histologischen Studien an den Nagezähnen des syrischen Goldhamsters soll eine kurze Betrachtung über den Schmelz und das Periodontium bilden. Auf Grund der geringen Bedeutung des Schmelzes für die experimentelle Kariesforschung halte ich eine umfassendere Darstellung nicht für erforderlich.

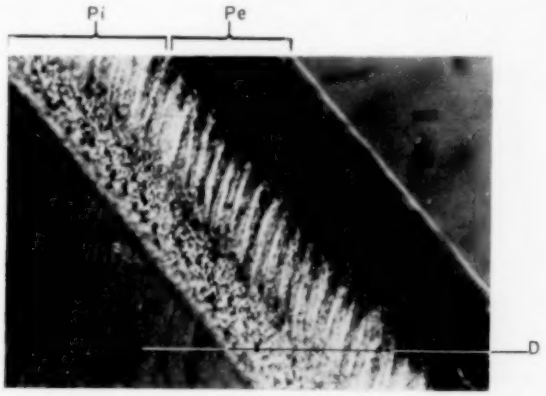


ABB. 16. Cricetus auratus, Radialschliff durch einen Nagezahn, Pi Partie interna, Pe Partie externa, D Dentin, gewöhnl. Licht, 600 : 1.

Gegenüber dem Hamstermolaren- und menschlichen Schmelz zeigt der Nagezahnschmelz nicht nur in topographischer Hinsicht eine völlige Abweichung, sondern auch bezüglich seiner feineren submikroskopischen Struktur. Histogenetisch finden wir die Ganoblasten auf der labialen Fläche des Zahnes palisadenförmig angeord-

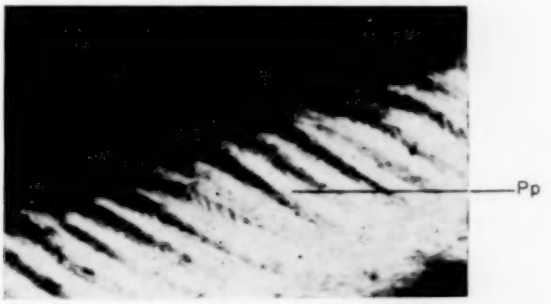


ABB. 17. Cricetus auratus, Radialschliff durch einen Nagezahn, Pp Pseudoprismen nach Korvenkontio, 900 : 1, Pol. +.

net. Die Strukturierung erfolgt nach Abscheidung der Fibrillen in die Grundsubstanz, die später bei Ablagerung von Kalksalzen die Kristalle orientieren. Die andersartige Struktur der Nagezähne ist vermutlich auf die naturbedingte hohe physiologische Beanspruchung der Nagezähne zurückzuführen.

KORVENKONTIO unterteilt den Schmelz in eine Außen- und Innenpartie (Partie externa und Partie interna). Die Partie interna zeigt beim Cricetus auratus auf ihrer basalen Fläche am Radialschliff kleine spaltenförmige, unregelmäßige Hohlräume,

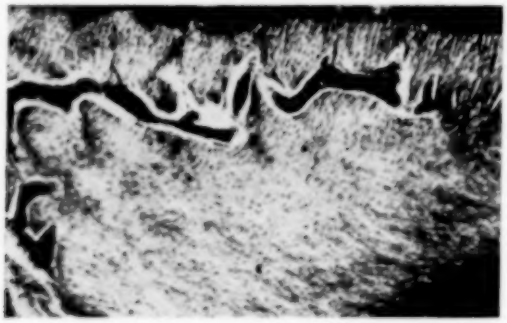


ABB. 18. Cricetus auratus, Radialschnitt durch das Periodontium eines Nagezahnes, girlandenförmig angeordnete Gefäße, 75 : 1, Bielschowsky-Färbung.

die zum größten Teil zwischen den Prismen eingelagert sind (Abb. 16). Die folgenden schräg liegenden parallelen Streifen werden nach KORVENKONTIO als Pseudoprismen bezeichnet, da es sich, wie man an Querschnitten feststellen kann, um

ABB. 19. Cricetus auratus, stärkere Vergrößerung aus Abb. 19, 150 : 1.

Querschnittsfiguren einzelner Lamellen handelt (Abb. 17). Das Vorzeichen der Prismen war bei allen untersuchten Nagezähnen hauptsächlich positiv zu ihrer Länge. Oft finden wir aber auch negativ doppelbrechende Bezirke innerhalb des Schmelzes vor.

Zum Periodontium ist noch kurz zu sagen, daß außer auf der labialen Seite auf dem übrigen Nagezahnteil, der sich im Knochen eingebettet befindet, eine hauchdünne Zementschicht liegt. Mikroskopisch läßt sich die Zementschicht nur durch einen schmalen Saum um den Zahn erkennen, der gegenüber dem Dentin ein ande-

Abb. 20. Cricetus auratus, Querschnitt durch einen Hamsterschädel, Nagezahn, starke Vaskularisierung des Periodontium, 45 : 1, He-Färbung.

res optisches Verhalten zeigt. Die elastischen Fasern verlaufen in der Richtung ihrer Beanspruchung und zwar stets in Zugrichtung. Imponierend ist im Periodontium die überaus starke Vaskularisierung, die sicherlich auch hier, ähnlich wie bei der Nagezahnpulpa auf das ständige Wachstum und den somit erhöhten Stoffwechsel zurückzuführen ist (Abb. 18, 19, 20). Einen weiteren Grund könnte noch die übermäßige Beanspruchung der Zähne während des Nageaktes bilden.

Vol. 6
1961

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 174–180, 1961. Pergamon Press Ltd.

CHANGES IN CRYSTAL TYPES OF LOW SOLUBILITY CALCIUM PHOSPHATES IN THE PRESENCE OF ACCOMPANYING IONS

H. NEWESELY

Forschungsgruppe für Mikromorphologie, Fritz-Haber-Institut,
Faradayweg 16, Berlin-Dahlem, Deutschland

Abstract—Hydroxy apatite $Ca_5(PO_4)_3$ OH is very stable in aqueous solutions under the conditions of a physiological milieu, but it is difficult to obtain pure hydroxy apatite crystals in such solutions. Under these biological conditions, with approximately neutral pH ranges and moderate temperatures, other calcium phosphates, e.g. tricalcium phosphate $Ca_3(PO_4)_2$ and octocalcium phosphate $Ca_4H(PO_4)_3.2H_2O$ may also occur. The specific course of the reaction is largely determined by small quantities of accompanying ions.

In the mineralization of teeth secondary constituents other than calcium and phosphate, present in tooth lymph, blood and saliva, are involved, especially in the form of Mg^{2+}, CO_3^{2-}, F^-, alkali ions, complex formers, as well as H^+ and OH^- in the concentration fixed by the pH value.

The influence on the types of crystallization is explainable by epitaxy. Moreover, uncertainties due to the colloid chemistry always occur in the formation of hard soluble calcium phosphates, as can be seen in the different behaviour of crystallized and precipitated (amorphous) material. Crystalline reaction products are only obtained with suitable concentrations of reaction agents and of the accompanying ions.

The results of *in vitro* crystallization experiments in the presence of some of the above-mentioned ions are reported.

For the formation of apatite structures under biological conditions the presence of small quantities of fluoride ions is necessary.

Zusammenfassung—Hydroxylapatit $Ca_5(PO_4)_3OH$ ist in wässrigen Lösungen bei den Bedingungen des physiologischen Milieus zwar sehr beständig; es ist aber schwierig, reine Hydroxylapatitkristalle in solchen Lösungen herzustellen. Unter diesen biologischen Voraussetzungen (annähernd neutraler pH-Bereich, mässig erhöhte Temperatur) können vielmehr auch andere Calciumphosphate, z.B. Tricalciumphosphat $Ca_3(PO_4)_2$ und Oktacalciumphosphat $Ca_4H(PO_4)_3.2H_2O$ entstehen. Der jeweilige Reaktionsverlauf wird dabei im wesentlichen von kleinen Mengen begleitender Ionen bestimmt.

Bei der Zahnmineralisation kommen für diesen Fremdioneneinfluss die Nebenbestandteile in Frage, die neben Calcium und Phosphat in Zahnlymphe, Blut und Speichel vorhanden sind (insbesondere Mg^{2+}, CO_3^{2-}, F^-, Alkaliionen, Komplexbildner, sowie H^+ und OH^- in der durch den pH-Wert bestimmten Konzentration).

Die Beeinflussung des Kristallisationstyps wird durch Epitaxie-Erscheinungen erklärt. Darüberhinaus taucht bei der Bildung der schwerlöslichen Calciumphosphate stets die kolloidchemische Problematik auf, die sich im unterschiedlichen Verhalten kristal-

lisierter und gefällter (amorpher) Stoffe äussert. Nur bei geeigneter Festlegung der Konzentration des Reaktionsmittels und der Begleitionen werden kristallisierte Reaktionsprodukte erzielt. Über unsere Ergebnisse von Kristallisationsversuchen *in vitro* in Gegenwart der genannten Ionen wird berichtet.

Für die Apatitstruktur ist bei biologischen Bedingungen die Gegenwart von kleinen Mengen Fluoridionen notwendig.

Vol. 6
1961

IN THE PAST, studies of the stable compounds in the calcium phosphate system ($Ca^{2+}/PO_4^{3-}/H_2O$) have been undertaken largely in experiments using pure chemical reagents. However, when considering the mineralization of tooth and bone, account must be taken of the fact that besides phosphate and calcium these tissues contain a series of secondary ingredients, especially Mg^{2+}, CO_3^{2-}, F^-, alkali ions, and complex formers.

This investigation will show how some of these secondary ingredients influence the crystallization of calcium phosphates:

(1) The pure ($Ca/PO_4/H_2O$) system contains in aqueous solutions Ca-ions and tertiary phosphate ions. These are in equilibrium with hydrogen phosphate and OH-ions according to the equation

$$PO_4^{3-} + H_2O \rightleftarrows HPO_4^{2-} + OH^- \qquad (1)$$

Therefore in the pure tertiary phosphate system a surplus of hydroxyl ions must be present, and the pH of such solutions is accordingly raised (pH > 12).

Under suitable conditions hydroxy apatite crystals $Ca_5(PO_4)_3OH$ can be built up of the Ca^{2+}, PO_4^{3-} and OH^- ions which are contained in the system. A detailed report of these conditions (e.g. hydrothermic reactions with Na^+ ions as mineralizator (HAYEK *et al.*, 1958) was given at the ORCA Congress in Pavia (NEWESELY, 1959).

(2) The pure system is only stable in the highly alkaline range. If the pH is lower, i.e. the solution is less alkaline, the equilibrium (1) is shifted to the right and the hydrogen phosphate ion appears at an increased concentration in the system. The presence of HPO_4^{--} ions disturbs the formation of pure hydroxy apatite and favours the formation of amorphous calcium phosphate precipitates of variable composition,

TABLE 1. TYPES OF CRYSTALLIZATION OF CALCIUM PHOSPHATES

(a) Hardly soluble	Ratio Ca:P	(b) More soluble
Hydroxy apatite $Ca_5(PO_4)_3OH$	5:3 = 1·67	Secondary calcium phosphate $CaHPO_4$
Tricalcium phosphate (water free) $Ca_3(PO_4)_2$	3:2 = 1·50	and $CaHPO_4.2H_2O$ 1:1 = 1,0
Octocalcium phosphate $Ca_4H(PO_4)_3.2H_2O$	4:3 = 1·33	Primary calcium phosphate $Ca(H_2PO_4)_2$ 1:2 = 0,5

with Ca/P atom ratios between 1.3 and 1.5, which therefore do not have the high Ca/P atom ratio of hydroxy apatite (1.67).

The heterogeneity is caused by adsorption phenomena and by such atomic disorders as the paracrystalline state. The precipitates therefore cannot be described by an exact stoichiometric formula. They are excluded here.

However, around neutrality a hard soluble crystalline reaction product $Ca_4H(PO_4)_3 . 2 H_2O$ octocalcium phosphate may also occur.

In a heterogeneous reaction by partial hydrolysis of secondary calcium phosphate $CaHPO_4.2H_2O$, this octocalcium phosphate can be prepared, but only as a microcrystalline product (HAYEK *et al.*, 1960). (Fig. 1).

FIG. 1. Octocalcium phosphate. × 7200.

Well-developed octocalcium phosphate crystals can be prepared by homogeneous crystallization from a buffered solution (pH = 6·5) at a slightly raised temperature (Fig. 2). The temperature required to initiate this reaction is undoubtedly dependent on the pH value of the solution, and decreases with a rise in the pH of the solution (Fig. 2a).

About 0.04 M [Ca] and 0.03 M [P] in 0·2 M sodium acetate buffer give a suitable ion concentration. As $CaHPO_4.2 H_2O$ is more soluble than octocalcium phosphate these concentrations are high enough to prevent contamination of the product with $CaHPO_4$. Results of analyses and structural data have already been published (NEWESELY, 1960), together with instructions for preparing this homogeneous crystallization. Octocalcium phosphate crystals are long thin foils (ruler-shaped small crystals). They are chemically and mechanically less stable than hydroxy apatite. (Fig. 3).

The crystal data of octocalcium phosphate, especially in comparison with hydroxy apatite, are now being investigated. Octocalcium phosphate is richer in energy (i.e. more able to react) than the apatite structure. Hence octocalcium phosphate is only stable in the above-mentioned conditions, and is sensitive to changes of pH

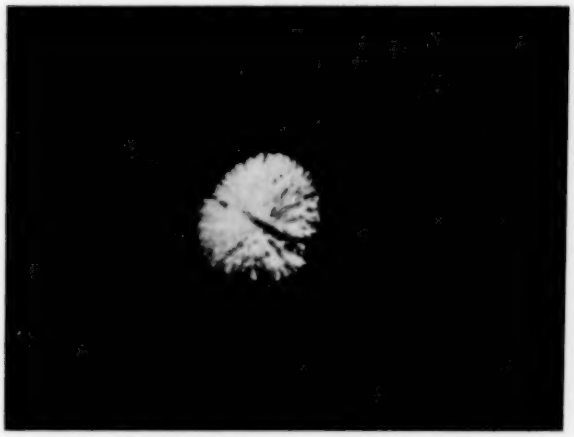


FIG. 2. Octocalcium phosphate. × 3.

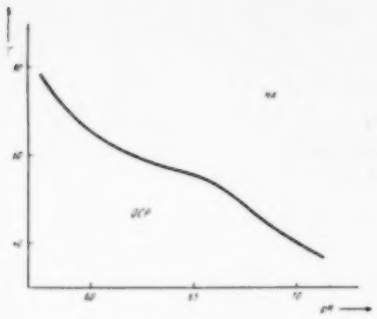


FIG. 2a.

and temperature. Octocalcium phosphate is converted into products with an apatite structure at higher pH's (i.e. with more hydroxyl ions) and at higher temperatures.

The tendency towards such structural conversions occurs not only with the addition of OH^- ions but also to an obvious degree in the presence of certain other accompanying ions.

(3) The effect of the magnesium ion and similar divalent ions (iron, manganese) is already known (HAYEK AND NEWESELY, 1958). It leads to the formation of the structure of the anhydrous tricalcium phosphate $Ca_3(PO_4)_2$ which otherwise is unknown in aqueous solution.

(4) Also in this connexion the effect of small quantities of fluoride in influencing the structure is especially noteworthy. In the presence of small quantities of fluoride (10–100 μg F^-/1000 ml) a reaction product with an apatite structure instead of octocalcium phosphate is formed immediately by homogeneous crystallization.

Octocalcium phosphate crystals which had previously been formed are converted into apatite in a heterogeneous reaction. The appearance of the apatite phase has been followed by X-ray diffraction and can without doubt be traced to the proceeding of the triplet-lines 321/310/402. This interesting phenomenon is an example of the effect of crystal germs in determining structure by epitaxy (the growing of crystal layers on a similar crystal grid).

TABLE 2. THE INDUCING OF THE APATITE STRUCTURE BY FLUORIDE IONS

Concentration F^- (μg/1000 ml)	X-ray diagram
0	OCP
10	OCP
20	Apatite?
100	Apatite

It is well known that fluoride and hydroxyl ions can substitute for each other isomorphously in the apatite lattice. In this interesting physiological milieu the concentration of OH ions is very low; the pH value 7 is equivalent to a $[OH^-]$ concentration of 10^{-7} mol/l, i.e. 1 μg OH^-/1000 ml. This concentration is much less than that necessary for the formation of apatite or even of apatite germs at physiological temperatures.

The level of fluoride needed for forming crystal germs of fluor apatite, i.e. fluor hydroxy apatite, may be calculated from the atomic weight of fluorine (19), the molecular weight of fluor apatite (1008), and the solubility of fluor apatite ($\sim$ 1 mg/1000 ml H_2O):

$$\frac{19}{1008} \cdot 1 \text{ mg} \cong 20\,\mu\text{g } F^-/1000 \text{ ml } H_2O \qquad (2)$$

This theoretical result corresponds closely to the value found by experiment of 10-100 μg of fluoride, which is sufficient to induce conversion.

The apatite structure of the crystal germs which is thereby made possible is impressed on the successive lattice planes. The necessary hydroxyl ions can be taken from the reservoir of the equilibrium

$$HPO_4^{--} + H_2O \rightleftarrows H_2PO_4^- + OH^- \qquad (3)$$

Thus in the formation of the crystal germ the fluoride concentration acts as a substitute for the higher pH value required for apatite formation. Formally the fluoride concentration can be expressed as a pF value similar to the pH value:

fluoride in drinking water: about 0·2 mg/1000 ml

$[F] = 0{\cdot}2 \text{ mg} = 200\,\mu\text{g} = 10^{-5}$ mol

$[F] = 10^{-5} \rightleftarrows \text{pF} = 5$

$$\text{pOH} = 5 \rightleftarrows \text{pH} = 9,$$
$$\text{as } H^+ \cdot OH^- = 10^{-14}\ (24°C) \qquad (4)$$

This fluoride concentration may be equated to the basic pH value, which lies far outside the range of stability of octocalcium phosphate and thus induces the apatite structure important for the normal tooth mineralization. In this context this phenomenon suggests that fluoride should be regarded as an essential trace element.

With reference to the detailed report on this problem by CREMER (1955) at the ORCA Congress in Geneva, the inducing of the apatite structure by fluoride ions underlines the importance of a sufficient quantity of fluoride being available at the earliest possible moment so as to influence the embryo.

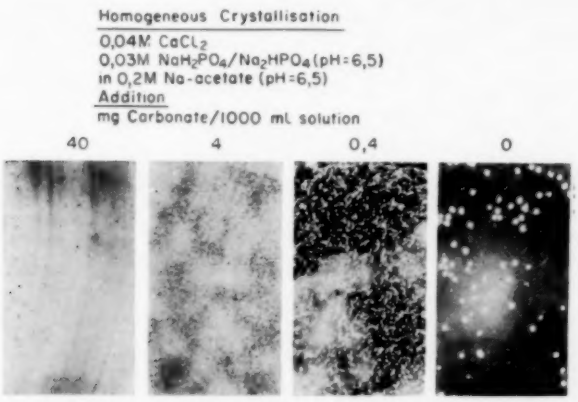


FIG. 3. Disturbing of crystal growth at the presence of Carbonate-ions.

The mineralization of the enamel matrix should not only be complete in quantity, but also correct in quality, i.e. as a fixed co-ordination of apatite crystals and the organic enamel matrix in the early state of mineralization. Other calcium phosphates are less well suited for this purpose (NEWESELY, 1961).

(5) It is not clear what influence, if any, the relatively high content of carbonate of the tooth (3–4 per cent) has on the apatite structure. According to the latest results (MCCONNELL, 1960), which, however, have not been carried out with monocrystals, carbonate is able to substitute for phosphate groups in the apatite lattice.

Changes of structure in the aqueous calcium phosphate system produced by accompanying carbonate ions are not found, but a content of more than 50 mg CO_3^{2-}/1000 ml disturbs the crystallization of calcium phosphate (Fig. 3). The increasing obstruction of growth is to be explained by the irreversible blocking of the growth layers of the phosphate crystals by adsorbed carbonate ions.

Such explanations stress the important part which accompanying inorganic ions may play in affecting the structure of mineralizing tissues in biological milieux.

Finally, I would like to emphasize that the heterogeneous results of the investigations of the chemical nature of tooth, calculus and nephritic stones can be explained by the influence of accompanying ions on their structure. Tooth, calculus and nephritic stones are said to contain hydroxy apatite and also tricalcium phosphate carbonate phosphate and octocalcium phosphate.

REFERENCES

HAYEK, E., BÖHLER, W. LECHLEITNER J. and PETTER H. 1958. Hydrothermalsynthese von Calciumapatiten. *Z. anorg. allg. Chem.* **295**, 241.

NEWESELY H. Herstellung synthetischer Hydroxylapatit-Einkristalle, Proceedings ORCA 6th Congress.

HAYEK, E., NEWESELY, H., HASSENTEUFEL, W. and KRISMER B. 1960. Zur Bildungsweise und Morphologie der schwerlöslichen Calciumphosphate. *Monatsh. Chem.* **91**, 249.

NEWESELY H. 1960. Darstellung von Oktacalciumphosphat durch homogene Kristallisation. *Monatsh. Chem.* **91**, 1020.

HAYEK, E. and NEWESELY H. 1959. Über die Existenz von Tricalciumphosphat in wässriger Lösung. *Monatsh. Chem.* **89**, 88.

CREMER, H. D.,: 1955. Die Bedeutung von Fluor im Rahmen der Spurenelemente. Proceedings ORCA 2nd Conference.

NEWESELY, H., 1961. Die Chancen und die Problematik der Zahnfluoridierung. *Die Pyramide* **9**, 1.

MCCONNELL, D., 1960. The crystal chemistry of dahllite. *Amer. Min.* **45**, 209.

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 181–192, 1961. Pergamon Press Ltd.

DER FLUORGEHALT VON TRINKWÄSSERN AUS VERSCHIEDENEN GEOLOGISCHEN FORMATIONEN THÜRINGENS

G. HENKEL

Klinik für Zahn-, Mund- und Kieferheilkunde der Friedrich Schiller Universität, Bachstrasse 18, Jena/Thür., Deutschland

Vol. 6
1961

Zusammenfassung—Seit 1955 werden in verschiedenen geologischen Formationen Thürigens die Fluorgehalte der Trinkwässer bestimmt. Es lagen bis Ende des Jahres 1960 weit über 3000 Analysen vor. Die Wässer entstammen der Buntsandsteinformation, dem Muschelkalk und dem Keuper. Weiterhin wurden zwei Gebiete mit Buntsandsteinformationen untersucht, die zu den Salzlagerstätten gehören.

Hydrologische Betrachtungen zeigen, daß größere Wasserreserven und Grundwasserführungen im Buntsandstein vorliegen. Muschelkalk- und Keuperschichten haben stark unterschiedliche Wasserführungen. Der Vergleich zwischen verschiedenen Quellen in den einzelnen Gebieten weist in einigen Fällen auf gleichmäßige Veränderungen des Fluorgehaltes hin. Die reinen Buntsandsteinformationen haben eine jährliche Fluorführung von 0,205 mg F pro Liter, die Muschelkalkformationen 0,320 mg F. Die Buntsandsteinformationen im Kalibergbaugebiet und im Solbadegebiet haben einen Durchschnitt von 0,380 bis 0,450 mg F. Trinkwässer aus der Saaleaue haben 0,145 mg F, das Keupergebiet dagegen 0,250 F. Eine fünfjährige Beobachtung in einem Muschelkalktal in der Nähe Jenas zeigt trotz großer Schwankungen zwischen den Analysen sehr gute Jahresdurchschnittswerte von 0,220 bis 0,240 mg F.

Neue Gebiete werden zur Zeit untersucht. Die kariesstatistischen Auswertungen werden später veröffentlicht.

DAS LAND Thüringen hat einen wechselvollen geologischen Aufbau. Es gleicht etwa einem Becken, dessen Ränder im Norden durch die Auffaltung des Harzes und im Süden durch die Auffaltung des Thüringer Waldes stark nach oben gebogen sind. Dadurch kommt es, daß die in diesem Becken meist horizontal übereinander gelagerten geologischen Schichten in den Randgebieten aufgewölbt wurden. So sind durch Verschiebungen, Aufstellungen und Verlagerungen der Gesteinsschichten Gebiete entstanden, die in ihrer Abwechslung nicht nur dem Geologen oder Mineralogen, sondern auch dem Hydrologen und Geographen, darüber hinaus aber auch dem Wanderer viele Anregungen und aufschlussreiche Beobachtungen bieten.

Als kleiner Ausschnitt aus solchen Beobachtungen wurden seit Jahren die Fluoraufkommen verschiedener Trinkwässer in unterschiedlichen geologischen Formationen Thüringens überprüft. Hier und da haben die Ergebnisse zum Teil in Verbindung mit den uns beschäftigenden Fragen des Kariesbefalls bereits Niederschlag in Veröffentlichungen gefunden (HENKEL, 1957, 1960, 1961; HOFFMANN, H., 1958;

HOFFMANN, U., 1958). Bisher ist es jedoch unterblieben, verschiedene Gebiete miteinander zu vergleichen. Da sehr zahlreiche Analysen durchgeführt wurden, die sich auch über längere Zeiträume erstreckten, konnte eine Übersicht erst jetzt zusammengestellt werden.

Die Untersuchungsergebnisse, die in diesem Bericht vorliegen, bauen sich auf etwa 3300 Einzelanalysen auf, die von 1955 bis zum Ende des Jahres 1960 gesammelt wurden. Die Untersuchungen laufen in einigen Gebieten weiter. Neue Gebiete sind bereits mit einbezogen. Über sie kann wegen der geringeren Zahl von Analysen zur Zeit noch nichts mitgeteilt werden.

Die Untersuchungen haben sich zunächst von Jena und seiner weiteren Umgebung aus entwickelt. Während in einer gut übersichtlichen Muschelkalkformation ein Teil des Trinkwasseraufkommens für Jena sehr sicher geprüft werden konnte, reizte zugleich die geologische Tatsache, daß mitten durch Jena Buntsandsteinformationen und Muschelkalkblöcke nebeneinander an die Oberfläche treten, zu weiteren Vergleichen. In der Umgebung Jenas bis zu einem Umkreis von 40 bis 50 km wurden darüber hinaus die Trinkwasseraufkommen sämtlicher Orte mehrfach überprüft und auf Fluor untersucht. Weiterhin sind in Jena und seiner nächsten Umgebung in der Saaleaue Brunnen angelegt worden, so daß hier Grundwässer alluvialer Schichten geprüft werden können, die dem Buntsandstein aufliegen.

Buntsandsteinformationen stehen darüber hinaus auch im westlichen Teil Thüringens, nämlich im Kalibergbaugebiet zwischen Bad Salzungen und Vacha, an.

Nördlich von Jena noch im Saaletal finden wir bekannte Solbadegebiete der Orte Bad Sulza und Bad Kösen. Auch hier wurden die Trinkwässer über ein Jahr lang regelmäßig untersucht. Vergleichende Ergebnisse wurden weiterhin aus dem um Erfurt gelegenen Keupergebiet zusammengetragen. Zahlreiche kleinere Ortschaften sind hierbei erfaßt worden, und nahezu 400 Analysen geben uns auch aus diesem Gebiet Aufschluß über das Fluoraufkommen von Trinkwässern in diesen Formationen.

Das Wasseraufkommen im allgemeinen in den verschiedenen geologischen Formationen Thüringens ist durch HOPPE (1952) näher untersucht worden. Seine Beschreibungen und Feststellungen dienen vor allem Fragen der Wasserversorgung der Bevölkerung und der Industrie. Nur für einzelne Gebiete liegen analytische Untersuchungen vor, die sich jedoch wenig oder fast gar nicht auf Spurenelemente beziehen. Trotzdem sind uns einige Hinweise, die die Wasserführung in den unterschiedlichen geologischen Schichten betreffen, wertvoll. Sie sollen kurz skizziert werden.

Unterer und mittlerer Buntsandstein

Er findet sich in den von uns untersuchten Gebieten der Werra (Westthüringen) und zwischen Saale und Elster (Ostthüringen) stark verbreitet (Abb. 1). Seine Aus-

bildung ist sehr unterschiedlich. Grobkörnige Schichten wechseln mit denen feineren Kornes ab, und auch die tonigen Bindemittel sind unterschiedlich. Der mittlere Buntsandstein tritt besonders im Gebiet von Jena und Kahla hervor. Im allgemeinen hat diese geologische Formation für die Wasserversorgung größte Bedeutung.

ABB. 1. Hydrogeologische Karte von Jena und Umgebung (HOPPE). ||||| = grundwasserarm (ob. Buntsandst., Röt; mittl. Keuper). ≡ = grundwasserreich, wechkalk, unter Keuper). ⋮ = Grundwasserführung stark wechselnd (Sandsteine). ≡ = grundwasserreich (unt. u. mittl. Buntsandst., ob. Keuper, Sandsteine).

Trotzdem ist in einzelnen Gebieten das Wasseraufkommen unterschiedlich. In der Nähe von Jena ist eine reichliche Wasserführung nachzuweisen. Die Wasservorräte auch nach längerer Trockenzeit sind bei den Buntsandsteinquellen ausreichend. Auf höhere Niederschläge reagieren sie erst nach längerer Zeit, oft nach Wochen. Die Buntsandsteingrundwässer sind in ihrer Qualität nicht so stark gleichbleibend

wie die Buntsandsteinquellen. Einen kleinen Einblick gewährt uns ein von HOPPE veröffentlichtes Profil durch das ostthüringische Buntsandsteingebiet (Abb. 2).

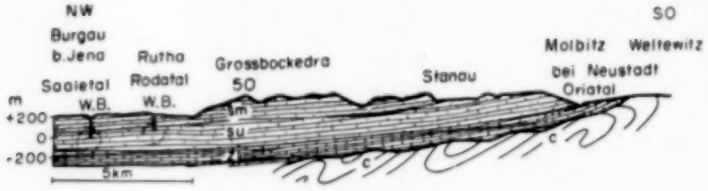


ABB. 2. Profil durch das ostthüringische Buntsandsteingebiet vom Schiefergebirge bis zum Saaletal bei Jena. Buntsandsteintiefbohrungen bei Burgau und im Rodatal. (HOPPE).

Oberer Buntsandstein oder Röt

Er ist für unsere untersuchten Quellen wichtig, da er unter dem klüftigen Muschelkalk gelegen ist und zu Quellhorizonten führt. An ihm treten die Quellen entweder unmittelbar oder unterhalb des Muschelkalkhangschuttes auf (Abb. 3, 4).

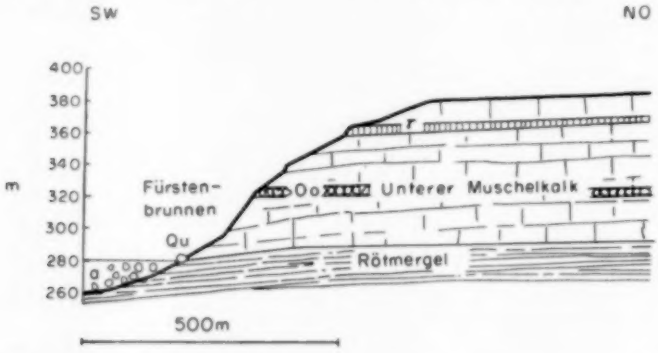


ABB. 3. Profil des Fürstenbrunnens von Wöllnitz bei Jena. Quellhorizont am Rötmergel (HOPPE).

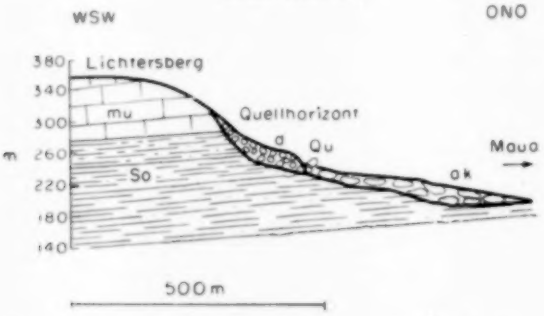


ABB. 4. Profil Lichtersberg-Mave, Hangschuttquelle (HOPPE).

Muschelkalk

Die Muschelkalkformationen Thüringens sind untereinander weitgehend ähnlich und weisen bei weitem nicht solche Unterschiede auf, wie es die Buntsandsteinformationen unter sich tun. Die Wasserführung wird von der physikalischen Beschaffenheit des Gesteins bestimmt und ist von mir eingehender in den Beobachtungen über die Fluorführung des Trinkwassers aus dem Mühltal bei Jena beschrieben

ABB. 5. Hydrogeologische Karte vom Keupergebiet (Vergl. Abb. 1) (HOPPE).

worden. Meist sind as Schichtquellen oder Hangschuttquellen, die uns das Wasser liefern. Die Wässer fliessen im Muschelkalk sehr rasch ab. Das Wasservorkommen geht in trockenen Zeiten außerordentlich stark zurück. Die von mir geprüften Wassermengen, die zu den Niederschlägen in Vergleich gesetzt wurden, decken sich mit denen von HOPPE beobachteten fast völlig.

Keuper

In unserer Beobachtungsreihe tritt er besonders im Thüringer Becken zutage (Abb. 5). Seine Formationen sind um Erfurt stärker entwickelt. Seine Schichten sind jedoch sehr unterschiedlich in ihrer Beschaffenheit. Meist finden wir sehr flach liegende Quellen, die auf undurchlässigen Keupermergeln ruhen. Sehr unsicher ist dadurch die Wasserversorgung in trockenen Zeiten. Die von uns untersuchten Orte leben bezüglich des Trinkwassers fast durchwegs von Quellen, während Grundwasserbohrungen nur für die Versorgung größerer Orte durchgeführt werden.

ABB. 6. Hydrogeolog. Karte vom Werragebiet (Vergl. Abb. 1) (HOPPE).

Alluvium

Aussagen über die Wasserführung in alluvialen Schichten sind sehr schwierig zu machen. Jeder Brunnen weist besondere Verhältnisse auf. Meist sind die durchflossenen Formationen sehr wechselhaft. In den Saaleauen stromaufwärts von Jena kommen die Trinkwässer mit den darunter liegenden Buntsandsteinschichten in

engste Berührung. Bei Wässern, die solchen alluvialen Schichten entstammen, ist nichts weiter über Spurenelemente ausgesagt.

Die folgenden Untersuchungsergebnisse sind sowohl nach Gebieten als auch nach Jahren zusammengefaßt. Es wurde das Monatsmittel der Fluorführung berechnet.

Das Fluoraufkommen der verschiedenen Brunnen in Werrakaligebiet ist nicht gleichmäßig (Abb. 6). Ein Vergleich der Trinkwässer von Merkers, Bad Salzungen,

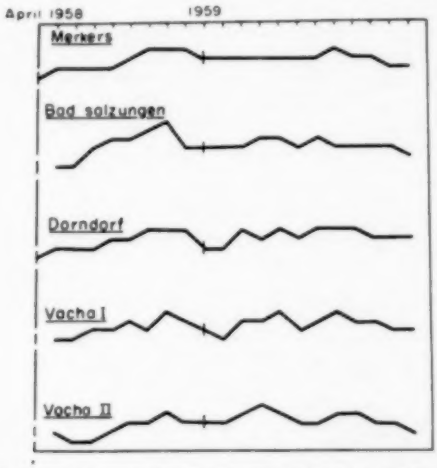


ABB. 7. Fluorführung der Trinkwässer im Kalibergbaugebiet.

Dorndorf, Vacha I, Vacha II, Hadra, Brunnen I und Brunnen III zeigt hin und wieder Zeiten gleichmäßigen Fluoranstieges oder auch -rückganges. Die Schwankungen sind offensichtlich nicht periodisch, sondern durch äußere Einflüsse bedingt.

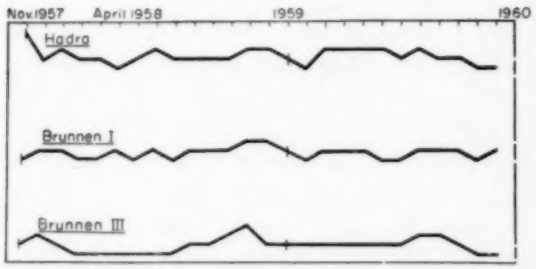


ABB. 8. Fluorführung der Trinkwässer im Kalibergbaugebiet.

Der Zeitpunkt solcher Veränderungen ist bei einigen Brunnen annähernd gleich (Abb. 7, 8).

Erneute Prüfungen des Fluorgehaltes der Mühltalquellen in Jena, die in der Muschelkalkformation liegen, läßt—wie schon früher festgestellt—jahreszeitliche Schwankungen erkennen. Die Unterschiede sind nicht sehr erheblich (Abb. 9).

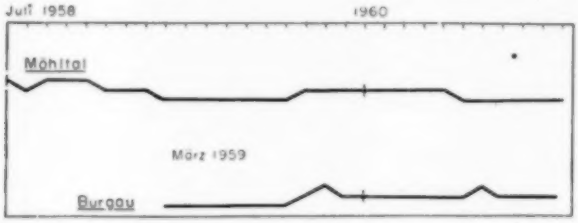


ABB. 9. Fluorführung der Trinkwässer im Muschelkalk vom Mühltal (Jena) und in der Saaleaue bei Burgau.

Die Analysen der Trinkwässer aus der Saaleaue bei Burgau ergeben einen ziemlich gleichmäßigen Fluorgehalt, der kaum beachtenswerten Schwankungen unterworfen ist (Abb. 9).

Die über einjährige Beobachtung im Keupergebiet zeigt im Frühjahr einen Abfall des Fluorgehaltes, der offenbar mit der im Sommer einsetzenden Trockenzeit wieder etwas ansteigt und dann über Monate hinaus gleichbleibt (Abb. 10).

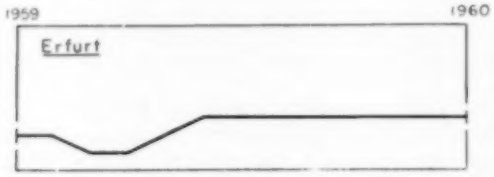


ABB. 10. Fluorführung der Trinkwässer im Keupergebiet.

Recht überraschende Ergebnisse zeigt das Solbadegebiet von Bad Sulza und Bad Kösen. Obwohl vier Quellen verschiedener Lage untersucht wurden, sind annähernd übereinstimmende Schwankungen in der Fluorführung festzustellen (Abb 11, 12).

Bei einem Vergleich der Fluorführung in den verschiedenen Untersuchungsgebieten ergeben sich sehr gute Durchschnittswerte. Wie am Anfang der Darstellung bereits hervorgehoben wurde, liegen diesen Durchschnittswerten rund 3300 Analysen zugrunde. In den nachfolgenden Übersichten sind außer den Untersuchungsgebieten noch die Jahre der Untersuchung und die Anzahl der Analysen vermerkt. Den niedrigsten Fluorgehalt weisen die Grundwässer der Saaleaue auf (Abb. 13). Er erreichte im Jahre 1959 den Durchschnittswert von 0.145 mg F/l. Durch Zuleitung weiterer Brunnenwässer, die am Rande der Saaleaue liegen, wurde 1960

der Durchschnittswert auf 0,190 mg F/l erhöht. Im Muschelkalkgebiet des Mühltales von Jena bleibt von 1955–1960 bei insgesamt 405 Analysen das Fluoraufkommen fast völlig gleich. Die Schwankungen der Werte können sehr wohl Beobachtungsungenauigkeiten bei der Analyse sein (Abb. 13). Auch das Keupergebiet um Erfurt bleibt mit 0,250 mg F/l dem Muschelkalkgebiet gleich. Auch hier liegen

ABB. 11. Hydrogeologische Karte vom Solbadegebiet Bad Sulza u. Bad Kösen (Vergl. Abb. 1) (HOPPE).

ausreichende Analysen, nämlich 378 vor (Abb.13). In den Jahren 1958 bis Ende 1959 wurden nun auch im Kalibergbaugebiet in 9 verschiedenen Orten laufend Trinkwasserproben entnommen. Die Gesamtzahl der Analysen ist 1559. Die einzelnen Wasserentnahmestellen, die sowohl Brunnen als auch Wasserleitungen betreffen, unterschieden sich nur sehr gering voneinander. Die Schwankungen bewegen sich von 0,380 bis 0,450 mg F/l (Abb. 14).

Zu gleichen Fluorwerten kamen wir bei den Trinkwasseranalysen im Solbadegebiet Bad Sulza und Bad Kösen. Die vier untersuchten Trinkwässer zeigen gleichen Anstieg oder Abfall in der Fluorführung. Der Fluorgehalt ist der gleiche wie in der Werraaue (Abb. 15).

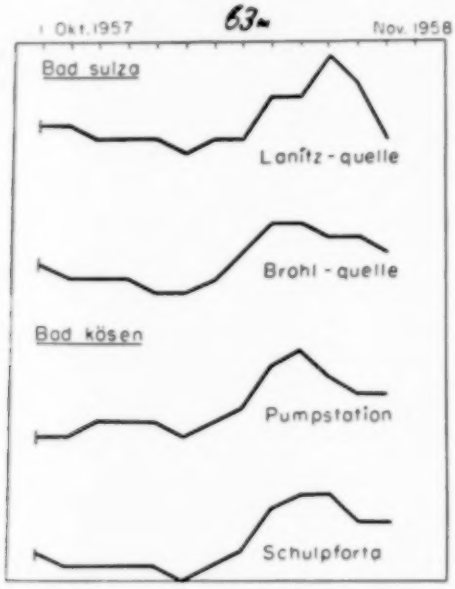


ABB. 12. Fluorführung der Trinkwässer im Solbadegebiet.

Muschelkalk			
Jena-Mühltal	1955–1956	(n = 106)	0,236 mg F/l.
	1957–1958	(n = 91)	0,244
	1959	(n = 208)	0,221
	1960		0,226
Saaleaue			
Jena-Burgau			
Tiefbrunnen	1959	(n = 97)	0,145
	1960	(n = 114)	0,190
Keuper			
Erfurt	1959–1960	(n = 378)	0,250

ABB. 13. Fluoraufkommen der Trinkwässer im Jahresmittel.

Die sehr hohe Anzahl der Analysen gibt uns eine weitgehende Sicherheit, daß die Fluorwerte in den einzelnen Gebieten nicht durch Zufälligkeiten beeinflußt sind. Wir können also die Ergebnisse als zuverlässig ansehen. Vergleichen wir die Messungen mit den hydrogeologischen Betrachtungen, wie sie uns HOPPE bietet,

Kalibergbaugebiet			
Werra-Aue + Buntsandstein			
Brunnen I	1958	(n = 235)	0,380 mg F/l.
	1959		0,380
Brunnen III	1958	(n = 205)	0,380
	1959		0,410
Dorndorf	1959	(n = 178)	0,410
Vacha I	1959	(n = 177)	0,450
Vacha II	1959	(n = 177)	0,430
Hadra	1958	(n = 233)	0,400
	1959		0,410
Salzungen	1959	(n = 178)	0,410
Merkers	1959	(n = 176)	0,400

ABB. 14. Fluoraufkommen der Trinkwässer im Jahresmittel.

Solbadegebiet		
Bad Sulza		
Lanitzquelle	(n = 104)	0,420 mg F
Brühlquelle	(n = 104)	0,420
Bad Kösen		
Pumpstation	(n = 104)	0,400
Schulpforta	(n = 104)	0,450

ABB. 15. Fluoraufkommen der Trinkwässer im Jahresmittel.

so finden wir einen Hinweis für die Erklärung des Fluoraufkommens. Muschelkalk und Keuper sind zum Teil verwandte Formationen. Der Buntsandstein weist andere chemische Zusammensetzungen auf. Sowohl im Solbadegebiet wie im Kalibergbaugebiet stoßen wir auf umfängliche Buntsandsteinformationen, die allerdings ihr Trinkwasser nicht im ursprünglichen Zustand liefern. Es machen sich hier offensichtlich Einflüsse der Salzlagerstätten bemerkbar. Vergleichen wir nämlich hierzu die Messungen in den Buntsandsteinformationen im Südosten von Jena, so finden wir bei 101 Analysen einen Durchschnittswert von 0,250 mg F/l, der also dem des Keupers und des Muschelkalkes im Mühltal bei Jena gleichkommt (Abb. 16).

Buntsandsteinformation		
Südosten von Jena	(n = 101)	0,250 mg F/l
Muschelkalkformation		
Nordwesten von Jena	(n = 157)	0,320

ABB. 16. Fluoraufkommen der Trinkwässer im Jahresmittel.

Das vorliegende Analysenmaterial gestattet uns Vergleiche zu ziehen, da die Untersuchungen sehr umfänglich sind. Wichtig ist, daß das Fluoraufkommen von Trinkwässern, die gut bekannten geologischen Formationen entstammen, gemessen werden konnte. Der Vergleich ist einwandfrei für die Muschelkalk-, Keuper-

Vol. 6 1961

und Buntsandsteinformation durchzuführen. Im Muschelkalk und Keuper sind im allgemeinen stärkere Schwankungen in der Wasserführung zu erwarten als im Buntsandstein, wo das Wasseraufkommen im Durchschnitt beständiger ist. Der Fluorgehalt ist im Muschelkalkgebiet etwas höher. Sehr auffallend ist der Einfluß anderer geologischer Formationen auf die Quellen und Brunnen des Buntsandsteins im Werra- und Solbadegebiet. Da längere Beobachtungszeiten vorliegen, können die Ergebnisse nicht zufällig sein. Der Fluorgehalt der Brunnen in der Saaleaue ist niedrig. Die mitgeteilten Untersuchungsergebnisse sollen dazu dienen, Vergleichswerte für andere geologische Formationen und andere Länder zu schaffen. Noch nicht abgeschlossen sind in den untersuchten Gebieten Thüringens die kariesstatistischen Auswertungen. Sie sollen später veröffentlicht werden.

LITERATUR

HENKEL, G. 1957. Der Einfluß der Wetterverhältnisse auf die Fluorführung von Trinkwässern. *Dtsche Stomatol.* **13**, 183.

HENKEL, G. 1960. Die Kariesanfälligkeit im Muschelkalk- und Buntsandsteingebiet im Vergleich zum Fluorgehalt der Trinkwässer. Verhandlungen der 5. Tagung der ORCA, *J. Dent. Belge*.

HENKEL, G. 1961. Trinkwasseranalysen und Karies im Kalibergbaugebiet Westthüringens. Verhandlungen der 7. Tagung der ORCA, *Arch. oral Biol.* **4**, 204–211.

HOFFMANN, H. 1958. Beziehungen zu dem Fluorgehalt des Trinkwassers und der Zahnkaries bei Schulkindern im Alter von 6–16 Jahren im Muschelkalkgebiet um Jena. Med. Diss., Jena.

HOFMANN, U. 1958. Beziehungen des Fluorgehaltes von Trinkwässern und sein Einfluß auf das kindliche Gebiß unter besonderer Berücksichtigung von Zeitpunkt und Dauer der Einwirkung. Med. Diss., Jena.

HOPPE, N. 1952. *Die Hydrogeologischen Grundlagen der Wasserversorgung in Thüringen*, Verlag Gustav Fischer, Jena.

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 193–197, 1961. Pergamon Press Ltd.

CHEMISCH-TECHNISCHE ERFAHRUNGEN DER TRINKWASSERFLUORIDIERUNG IN KARL-MARX-STADT

II. BERICHT

H. LINGELBACH

Bezirks- Hygiene- Institut, Wasserhygiene, Herwegstrasse 3, Karl-Marx-Stadt, Deutschland

Vol. 6
1961

Zusammenfassung—Seit der Inbetriebnahme der Fluoridierungsanlage wurde eine Reihe von chemisch-technischen Erfahrungen gesammelt, wodurch Mängel behoben und Verbesserungen in der Dosierung vorgenommen werden konnten. Die Dosierung wird durch unterschiedliche Fluorwerte im Rohwasser, durch den wechselnden Na_2SiF_6-Gehalt der Chemikalie und durch Löslichkeitsschwierigkeiten beeinflusst. Der F'-Gehalt des Rohwassers aus den verschiedenen Talsperren schwankt zwischen 0,05 und 0,35 mg/l, der Na_2SiF_6-Gehalt liegt zwischen 88 und 95 Prozent. Löslichkeitsschwierigkeiten ergaben sich unter anderem durch das Abwerfen der Chemikalie in den Lösebehälter und durch schwankende Temperaturen des Lösewassers. Eine Aufheizung des Lösewassers wäre mit einer Erhöhung der Fluoridierungskosten bis zu 110 Prozent verbunden und wurde als unwirtschaftlich verworfen. Sämtliche chemisch-technischen Schwierigkeiten der Dosierung konnten inzwischen soweit behoben werden, dass sich der Fluorgehalt des Trinkwassers zwischen 0,9 und 1,1 mg/l bewegt. Die Gesamtkosten der Trinkwasserfluoridierung liegen bis jetzt bei 7,7 Dpf pro Kopf und Jahr der Bevölkerung. Im Zusammenhang mit der Dosierung stehende Korrosionsfragen konnten durch den Einsatz geeigneter Materialien gelöst werden. Eine befürchtete Rostabtragung im städtischen Wasserrohrnetz, sowie "Fluorzehrungen" wurden bis jetzt nicht festgestellt.

Abstract—Since the fluoridation plant came into use, a number of chemical and technical experiences have been obtained by which deficiencies can be removed and improvements in dosing made. The dosing depends on the various fluorine values of the water in the pipes, on the variable Na_2SiF_6 content of the chemical and on difficulties with regard to solubility. The F'-content of the piped water from various sources moves between 0·10 and 0·35 mg/l. while the Na_2SiF_6 content lies between 88 and 95 per cent. Difficulties with regard to solubility were found in several ways such as through dropping the chemical into the solution vessel and through the varying temperatures of the water used for solution. Raising the temperature of the water in which the chemical has to dissolve would cause the cost of fluoridation to increase by 110 per cent; it was rejected as an uneconomical proposition. The chemical-technical difficulties of giving the appropriate dose could meanwhile be solved in so far as the fluorine content of the drinking water lies between 0·9 and 1·1 mg/l. At the present moment the total expense of the fluoridation of drinking water is 7·7 German Pfennig (roughly 1½d.) annually per head of the population. Problems of corrosion in connexion with the dosing could be solved through the use of appropriate materials. It was feared that rust would show up in the water pipes of the town and that the pipes would show damage through fluorine application; so far however neither have been observed.

SEIT $1\frac{1}{2}$ Jahren wird das gesamte Trinkwasser der ca. 300 000 Einwohner zählenden Stadt Karl-Marx-Stadt fluoridiert. Während dieser Zeit wurden durch das Sammeln einer Reihe chemisch-technischer Erfahrungen verschiedene Mängel in der Fluordosierung aufgedeckt und behoben. Dadurch konnte die Durchführung der Trinkwasserfluoridierung wesentlich verbessert werden.

Karl-Marx-Stadt erhält sein Trinkwasser aus vier Talsperren, deren Fluorgehalte unterschiedlich sind und außerdem in sich schwanken. Der Schwankungsbereich dieser Talsperrenwässer liegt zwischen 0,05 und 0,35 mg/l Fluor-Ion. Die Nutzung der einzelnen Talsperren erfolgt nach wasserwirtschaftlichen Gesichtspunkten und zwar so, daß bezüglich der Trinkwasserfluoridierung im günstigsten Falle über einen Zeitraum Wasser aus einer Talsperre entommen wird, im ungünstigsten Falle dagegen Mischwasser aus allen vier Talsperren. Durch häufige Kontrollen des Fluorgehaltes des zur Aufbereitung kommenden Rohwassers, besonders dann, wenn die Mischungsverhältnisse der einzelnen Talsperrenwässer verändert werden, wird die Möglichkeit von Dosierungsschwankungen, hervorgerufen durch den unterschiedlichen Fluorgehalt des Rohwassers, weitgehend ausgeschaltet. Eine weitere Fehlerquelle für die Dosierungsgenauigkeit stellt der schwankende $Na_2Si\,F_6$-Gehalt der zur Verwendung kommenden technischen Natriumsilikofluorid-Chemikalie dar. Bei der Einzelüberprüfung des Inhaltes sämtlicher angelieferten Chemikaliensäcke wurde festgestellt, daß der $Na_2Si\,F_6$–Gehalt zwischen 88 und 95 Prozent schwankte. Rückfragen im Herstellerbetrieb ergaben, daß die Chemikalie in Chargen von 200 kg hergestellt wird und jeweils einige Chargen beim Trocknungsprozess gemischt werden. Dadurch werden größere Schwankungen im $Na_2Si\,F_6$-Gehalt der Chemikalie teilweise ausgeglichen. Die Schwankungen des Fluorgehaltes im Rohwasser und in der Chemikalie erschweren eine genaue Fluordosierung, da die Fluorwerte im Trinkwasser bei gleichbleibender Einstellung der Dosierungsmaschine zwischen 0,85 und 1,15 mg/l liegen können, wie die in diesem Zusammenhang durchgeführten Messungen zeigten. Die Anpassung der Dosierung an die veränderlichen Fluorgehalte des Rohwassers und der Dosierungslösung konnte durch entsprechendes Umstellen der Dosierungsmaschine erst nachträglich erfolgen, nachdem der Fluorgehalt des Trinkwassers im Laboratorium ermittelt worden war. Um zur Verhinderung von Fluorschwankungen im Trinkwasser kurzfristig Umstellungen der Dosierungsmaschine vornehmen zu können, wird seit einiger Zeit von dem Meister, der die Maschine bedient, der Fluorgehalt des Rohwassers, der Dosierungslösung und des fluoridierten Wassers in kurzen Zeitabständen an Ort und Stelle gemessen. Für diese Betriebskontrolle wird das Zirkon-Alizarin-Verfahren verwendet. Die Messungen erfolgen mit einer Vergleichsreihe in Neßlerzylindern. Nach einer Stunde Reaktionszeit können einwandfrei in einem Messbereich von 0–1,3 mg/l Fluor-Ion Konzentrationsunterschiede von 0,05–0,1 mg/l ohne optische Hilfsmittel erkannt werden. Das Zirkon-Eriochromcyanin-Verfahren, das sich für diese Art der

visuellen Betriebsmessung nicht eignet, und für die Überwachung im Hygiene-Institut sowie im Wasserwirtschaftsbetrieb verwendet wird, stimmt hinsichtlich der Meßgenauigkeit mit der Zirkon-Alizarin-Betriebskontrolle völlig überein. Die an Hand dieser Betriebskontrolle ermittelten Fluorwerte des Rohwassers und der Fluordosierungslösung werden auf einem Nomogramm für die in Abhängigkeit von den Fluorgehalten des Rohwassers und der Dosierungslösung einzustellende Dosierungsmaschine ausgewertet.

Eine andere Fehlerquelle wurde hinsichtlich der Beeinträchtigung der Dosierungsgenauigkeit in der differierenden Konsistenz der Chemikalie entdeckt. Die Konsistenz des zur Anwendung kommenden Natriumsilikofluorids schwankt zwischen staubfeiner und feinkörniger Beschaffenheit. Während die staubfeine Chemikalie zur Brückenbildung neigt und teilweise durch Beklopfen der Bunkerwände in die Wägevorrichtung gebracht werden muß, floß das körnige Material nach Ausschalten des Antriebsmotors weiter durch die Förderschnecke in den Wägebehälter und verhinderte die exakte Einhaltung des vorgesehenen Chemikaliengewichtes zur Herstellung der Dosierungslösung. Durch Einbau einer verstellbaren Ablenkvorrichtung wurde der Fördermechanismus auf die jeweilige Konsistenz des Natriumsilikofluorids abgestimmt.

Die Zugabe der Chemikalie in den mit Wasser gefüllten Lösebehälter erfolgt durch das Öffnen der Bodenklappe des Wägebehälters. Zur Beschleunigung des Lösevorganges sind Pumpen eingesetzt, die den Inhalt des Lösebehälters in Umlauf halten. Da trotz der Wasserumwälzung das Natriumsilikofluorid rasch zu Boden sinkt und einen relativ festen Belag bildet, der sich schlecht löst, wird die Chemikalie vom Wägebehälter in ein Gazebehälter entleert, der sich an der Wasseroberfläche im Lösebehälter befindet und nur wenig eintaucht. Das Pumpen bewirkt eine intensive Wasserbewegung, wodurch nach und nach die Chemikalie aus dem Gazebehälter herausgewaschen wird. Durch die Lagerung entstandene Klumpen werden während des Lösevorganges an der Gazewand zerrieben, so daß sich nur die unlöslichen Chemikalienbestandteile am Boden des Lösebehälters absetzen können.

Da es trotzdem noch teilweise zum Absetzen von Natriumsilikofluorid am Boden des Lösebehälters kam, wurde damit begonnen, zur weiteren Unterstützung des Lösevorganges mittels eines Kompressors Luft dicht über dem Boden des Lösebehälters in die Dosierungslösung einzudrücken. Dadurch wird der gesamte Behälterinhalt in intensive Wallung versetzt und eine gute Durchmischung der Lösung ermöglicht.

Ein wesentlicher Faktor zur Erhaltung einer genauen Fluordosierung im Trinkwasser stellt die Konstanthaltung der Konzentration der Dosierungslösung dar. Nachdem durch technische Verbesserungen in der Fluoridierungsanlage der Lösungsvorgang wesentlich verbessert wurde und die zur Verwendung kommende 0,4 prozentige Dosierungslösung im Lösungsbereich der niedrigsten Talsperrenwasser-

temperaturen liegt, ist es möglich, Schwankungen des Fluorgehaltes im Trinkwasser auf ein Minimum zu beschränken. Die Schwankungen, die noch ± 10 Prozent betragen, werden durch intensive chemische Kontrollen des Rohwassers, der Chemikalie und der Dosierungslösung an einen Schwankungsbereich von ± 5 Prozent herangeführt, das heißt, der Fluorgehalt im Karl-Marx-Städter Trinkwasser liegt dann zwischen 0,95 und 1,05 mg/l. Wäre zur Verbesserung der Löslichkeit des Natriumsilikofluorids anstelle der erwähnten technischen Verbesserungen und der Herabsetzung der Konzentration der Dosierungslösung eine Aufheizung der Lösung erfolgt, so hätte dieses eine wesentliche Erhöhung der Kosten der Trinkwasserfluoridierung zur Folge gehabt. Wirtschaftliche Untersuchungen ergaben, daß das Aufheizen um 5°C zusätzliche Kosten von 15,95 DM/d
um 10°C zusätzliche Kosten von 31,90 DM/d
um 15°C zusätzliche Kosten von 47,85 DM/d verursachen würden.

Geht man davon aus, daß die Temperaturen des Talsperrenwassers, wie bereits bei der Trinkwasserfluoridierung festgestellt wurde, im Winter teilweise auf 2°C absinken und die maximale Löslichkeit des Natriumsilikofluorids bei 17°C liegt und 0,65 Prozent beträgt, dann müßte im ungünstigsten Falle zur Erreichung der maximalen Löslichkeit eine Aufheizung des Lösewassers um 15°C erfolgen. Bei den ermittelten täglichen Regiekosten für die Trinkwasserfluoridierung von 43,40 DM würde die Aufheizung des Lösewassers um 15°C eine Kostenerhöhung der Fluoridierung um 110 Prozent zur Folge haben. Aufheizungen der Dosierungslösung um 5 bis 15°C würden mit Kostenerhöhungen zwischen 37 und 110 Prozent verbunden sein, die jedoch nicht notwendig sind, da, wie bereits erwähnt, durch technische Verbesserungen und niedrige Konzentrationen eine gute Löslichkeit des Natriumsilikofluorids erreicht werden kann.

Die veranschlagten Kosten der Trinkwasserfluoridierung von 10 Dpf pro Kopf und Jahr der Bevölkerung wurden wesentlich unterschritten und lagen bis jetzt bei 7,7 Dpf. In diesem Betrag sind Reparaturarbeiten mit enthalten, die bei Beginn der Fluoridierung auftraten, so wie alle anderen Kosten, die direkt oder indirekt mit der Fluoridierung in Verbindung standen. Aus den äußerst niedrigen Kosten kann geschlossen werden, daß die Karl-Marx-Städter Trinkwasserfluoridierung im Vergleich zu anderen Städten, eine der billigsten in der Welt darstellt.

Eine Gefahr der Störung des Betriebsablaufes in der Fluoridierungsanlage stellt die starke Aggressivität der Natriumsilikofluoridlösung dar. Die Dosierungslösung, die einen pH-Wert zwischen 3 und 4 aufweist, hat bereits mehrmals im Hinblick auf Materialzerstörungen große Schwierigkeiten bereitet. Sämtliche Eisenleitungen wurden trotz ihres intensiven Schutzanstriches bereits nach einigen Monaten unbrauchbar und mußten durch Kunststoffleitungen ersetzt werden. Diese sind inzwischen ungefähr 1 Jahr in Betrieb und haben sich gut bewährt. Weitere Materialzerstörungen traten dort auf, wo die Geschwindigkeit der Dosierungslösung hoch

Vol. 196

liegt. So wurden unter anderem Messingarmaturen innerhalb eines Jahres funktionsunfähig.

Der aus Bronzegewebe zusammengelötete Gazebehälter im Lösungsbehälter mußte bereits nach einigen Wochen gegen einen Behälter aus Perlongaze ersetzt werden, da sich der Lötzinn der Nähte vollkommen aufgelöst hatte und auch das Bronzegewebe deutlich Korrosionserscheinungen aufwies. Die aus Stahlbeton bestehenden Löse- und Dosierungsbehälter weisen bis jetzt keinerlei sichtbare Zerstörungen auf, obwohl diese Behälter nur einen Zementglattstrich besitzen und keinerlei Korrosionsschutz durch Anstrich erhalten haben.

Bedenken, die dahin gingen, daß durch das Fluor im Trinkwasser ein Abtragen des Rostes aus dem städtischen Rohrnetz erfolgt, haben sich bis jetzt nicht bestätigt, da laufende Eisenkontrollen des Trinkwassers keine Erhöhungen des Eisengehaltes zeigen. Bei den Untersuchungen des Fluorgehaltes im Trinkwasser des gesamten Stadtgebietes konnten bis jetzt keine "Fluorzehrungen" festgestellt werden.

Nach $1\frac{1}{2}$ jährigem Betrieb der Fluoridierungsanlage kann festgestellt werden, daß sämtliche chemisch-technische Mängel erkannt und beseitigt wurden, wodurch eine kontinuierliche Fluordosierung von 1 mg/l mit Abweichungen von $\pm$ 5 Prozent möglich ist.

Vol. 6
1961

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 198–202, 1961. Pergamon Press Ltd.

CARIES FREQUENCY AND FLUOROSIS IN MALLNITZ AND THE VALLEY OF ÖTZ (1·0–1·8 p.p.m. F IN THE DRINKING WATER)

K. BINDER

School Dental Service of Vienna, Schubertgasse 23, Vienna, Austria

Abstract—In two areas of Austria the drinking water shows a natural fluorine content of 1·0-1·8 p.p.m. (Mallnitz has 1·0 p.p.m., some places in the Ötz and Inn Valleys have 1·5–1·8 p.p.m.). In these places all children aged 6–14 were examined (400 boys and girls). Furthermore all children (377) of neighbouring communities in which the drinking water has a very low F-content were also examined. In all places with a poor F-content of drinking water, caries affections were practically the same, while the places with high F in the drinking water showed less caries in milk as well as permanent teeth at a rate dependent on the F-content of the water, namely at 1·8 p.p.m. it was less than at 1·5 p.p.m., and again at the latter rate much less than 1·0 p.p.m. As a protection against caries, milk teeth need considerably more fluorine than permanent teeth. At 1·0 p.p.m. the fluorine offers relatively little protection to milk teeth, at 1·5 p.p.m. the protection is very much higher, and at 1·8 p.p.m. it is 2·7 times as high as at 1·0 p.p.m.

At 1·0 p.p.m. F 18·7 per cent of the children showed fluorosis, at 1·5 p.p.m. the percentage was 40 and at 1·8 p.p.m. fluorosis was observed in 59 per cent of the children. Not only the number but also the degree of fluorosis showed greater intensity.

Zusammenfassung—In Österreich weist das Trinkwasser in zwei Gegenden einen natürlichen F-Gehalt von 1,0 p.p.m. bis 1,8 p.p.m. auf. (Mallnitz - 1,0 p.p.m., einige Orte im Ötztal und im Inntal–1,5–1,8 p.p.m.) Alle 6–14 jährigen Kinder wurden in diesen Orten untersucht (400 Knaben und Mädchen). Ferner wurden in Nachbarorten, die sehr niedrigen F-Gehalt des Trinkwassers haben, ebenfalls alle Kinder (377) untersucht. Während der Cariesbefall in allen F-armen Orten praktisch derselbe war, zeigte er sich in den F-Orten sowohl im Milch- als auch im bleibenden Gebiss abhängig vom F-Gehalt des Trinkwassers und war bei 1,8 p.p.m. geringer als bei 1,5 p.p.m. und hier wieder sehr wesentlich geringer als bei 1,0 p.p.m. Milchzähne brauchen zur Erlangung eines Cariesschutzes wesentlich mehr Fluor als bleibende Zähne. Bei 1,0 p.p.m. ist der Cariesschutz im Milchgebiss noch relativ gering, bei 1,5 p.p.m. ist er wesentlich höher, bei 1,8 p.p.m. ist er 2,7 mal so hoch als bei 1,0 p.p.m.

Während bei 1,0 p.p.m. F 18,7 Prozent der Kinder Fluorosen aufwiesen, waren es bei 1,5 p.p.m. 40,0 Prozent und bei 1,8 p.p.m. 59,0 Prozent. Die Fluorosen nehmen nicht nur zahlenmässig, sondern auch in der Stärke zu.

IN AUSTRIA there are two regions with a high natural content of F in the drinking water, one in Carinthia: Mallnitz (1·0 p.p.m. F) another in Tyrol, namely Silz in the valley of the river Inn (1·5 p.p.m.F) and Umhausen in the valley of the river

Ötz (1·8 p.p.m. F). On the instigation of the Austrian Ministry of Health an investigation into dental health was started in those regions and carried out in cooperation by the Institute of Hygiene of the University of Innsbruck (Director: Prof. Dr. A. SCHINZEL), the Dental Clinic of the same University (Director: Prof. Dr. O. PREISSECKER) and myself.

Of these investigations, concerning the incidence of caries and the frequency of fluorosis in the above-named regions, I should like to present to you here the preliminary results, while our inquiries are continuing.

Our first problem was to find for every group living in a region with high F-content in its drinking water a corresponding control group with a low F-content in its water supply. On examination, the tapwater in Mallnitz showed a concentration of 1·0 p.p.m. F, while the water of all the private wells in the village showed merely traces of F. Since only one half of the houses are connected with the municipal water supply, while the rest of the population takes its water from private wells, we found the ideal group for comparison in Mallnitz itself.

Vol. 6
1961

TABLE 1. NUMBER OF CHILDREN IN THE DIFFERENT GROUPS

	Ø F-group	F-group	Total
Mallnitz	49	64	113
Tyrol	328	336	664
Total	377	400	777

A similar situation does not occur in Tyrol. Therefore we had to use villages in the neighbourhood for comparison with ours. The water of those villages—Längenfeld, Huben, Mötz and Miming, contained only traces of F. All the children in these villages in the age group of 6-14 years have been examined. Their numbers in the different groups can be seen in Table 1. The caries incidence and frequency of fluoroses are shown in Table 2.

TABLE 2. INCIDENCE OF CARIES AND FLUOROSES

	Mallnitz Ø F	Tyrolia Ø F	Mallnitz 1·0 p.p.m.F	Silz 1·5 p.p.m.F	Umhausen 1·8 p.p.m.F
DMFT per child	2·55	2·77	1·25	0·52	0·39
dmft per child	4·18	4·12	3·60	2·13	1·67
% of T carious*	17·4	17·7	8·3	4·1	2·9
% of t carious	43·3	47·0	38·1	22·4	15·9
% of caries-free children (DMFT = Ø)	22·4	24·7	46·8	72·9	81·1
Mottled enamel (%)	0·0	0·0	18·7	40·0	59·0

* T = total number of all permanent teeth;
t = total number of all deciduous teeth.

As the caries incidence in the four Tyrolian villages with the low F-content of the drinking water was remaining nearly on the same level, we could treat the findings in the four groups together as a single group (see Table 2, Tyrolia Ø F).

The incidence of caries in the permanent teeth of the control group in Mallnitz was slightly lower than in Tyrol. The reason for this may be seen in the fact that the children, although getting their drinking water at home from private wells, had access to tapwater from the municipal supply at school or at the homes of friends and received therefore more F than the children of the Tyrolian control group. This fact will be treated later on.

DISCUSSION OF THE RESULTS

(1) The fact that the incidence of caries was nearly the same in all control groups and decreased with increasing F-content of the drinking water, proves that the F is the only reason for the decrease of the frequency of caries. Furthermore, when the Austrian Ministry of Health ordered an inquiry on the subject of caries frequency in all Austrian counties, the results were practically the same as shown by our control groups with low F-level. A caries incidence as low as in the F-districts could not be found anywhere else in Austria.

(2) At 1·5 p.p.m. F there could be found only half as many carious lesions as at 1·0 p.p.m. F in permanent teeth. At 1·8 p.p.m. F they were still less, but the difference of the carious lesions between 1·8 and 1·5 p.p.m. was much less than that between 1·5 and 1·0 p.p.m.

(3) The protection from caries by water rich in F was much less pronounced for the deciduous teeth than for the permanent ones. At 1·0 p.p.m. F the caries protection of the deciduous teeth is comparatively low. It is higher at 1·5 p.p.m. but at 1·8 it is 2·7 times as efficient as at 1·0 p.p.m. This means that the deciduous teeth need much more F for prevention of caries than the permanent ones.

(4) Corresponding to the increasing F-content of the drinking water there was an increase of the fluoroses to be observed, not only in number, but also in severity. At Mallnitz (1·0 p.p.m. F) white opacities were to be found and light yellowish staining existed only in a few cases. The mottling was not noticeable except to a trained observer. (Questionable and very mild mottling according to the classification of Dean). At Silz (1·5 p.p.m.) brown staining predominated. At Umhausen (1·8 p.p.m.) in some cases marked hypoplasia and pitting occurred. The brown staining was darker and widespread. We had the impression that the mottling, especially at Umhausen but also at Silz, was more severe and more frequent than we had expected from the international literature. This will be the subject of further examinations.

(5) As the caries protection at 1·5 p.p.m. is much more pronounced than at 1·0 p.p.m. it should be a matter of further discussion whether or not it would be

recommendable to increase the F-content to a higher level than 1·0 p.p.m. in artificial water fluoridation.

Furthermore we were interested in the question of whether there were significant differences between girls and boys. (see Table 3).

TABLE 3. CARIES INCIDENCE—GIRLS AND BOYS SEPARATED

	Mallnitz Ø F		Mallnitz 1·0 p.p.m. F		Umhausen 1·8 p.p.	
	♂	♀	♂	♀	♂	♀
DMFT per child	2·38	2·83	1·10	1·48	0·37	0·40
dmft per child	4·48	3·66	3·82	3·28	1·91	1·46
% of T carious	16·29	19·31	7·43	9·61	3·02	2·87
% of t carious	45·72	31·93	38·40	37·61	17·19	14·71
% of caries-free children (DMFT = Ø)	29·03	11·11	53·84	36·00	84·14	78·49

Table 3 shows that there is no marked difference of the F-action in girls and boys. The DMF in girls is higher than in boys, with only one exception. The boys at Umhausen show more carious lesions in their permanent teeth than the girls, but only when expressed in percent of all present permanent teeth. The percentage of caries-free children is again higher in boys than in girls. The reason for this exception is the small number of children examined. I should like to draw your attention to the fact that the boys' deciduous teeth are worse than the girls'. We found the same result at an inspection at Vienna, when we made a trial with fluor tablets. This is a contradiction for which we had no explanation at first, but we found one later.

TABLE 4. CARIES INCIDENCE IN MALLNITZ, AGE-GROUPS SEPARATED

Ø F-group	6–10 years		11–14 years	
	Ø F-group	F-group	Ø F-group	F-group
DMFT per child	1·6	0·9	4·3	1·8
dmft per child	5·7	4·5	1·0	1·7

At Mallnitz we separated the children in different age-groups (see Table 4). This table shows the surprising fact that the elder children in the F-group have much more carious deciduous teeth than in the control group in contradistinction to the condition in the permanent teeth and also to the condition in the younger children. The explanation is that the deciduous molars in the control group of the elder children have been lost earlier and that the permanent premolars have erupted earlier than in the F-group. If this explanation were true, there had to be more

permanent teeth present in the control group than in the F-group, and less deciduous teeth in the control group than in the F-group, which in fact we could prove (see Table 5).

TABLE 5. NUMBER OF PRESENT PERMANENT AND DECIDUOUS TEETH IN MALLNITZ

	Ø F-group	F-group
Deciduous teeth per child	2·0	3·0
Permanent teeth per child	24·6	23·1

The explanation for this relatively earlier eruption of the premolars in the control group could be a retardation of the shedding in the F-group. But there is still another reason. The children of the control group had worse teeth than the others so they visited the dentist more frequently, who extracted the carious deciduous molars. It is well known that after early extraction of the deciduous molars the premolars erupt earlier.

And so we found the explanation of why boys have worse deciduous teeth than girls, in contrast to the permanent teeth. The shedding occurs earlier in girls than in boys and the girls, having worse teeth than the boys, visit the dentist more frequently and consequently have their deciduous molars extracted earlier.

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 203–208, 1961. Pergamon Press Ltd.

DER FLUORIDGEHALT MONTENEGRINISCHER TRINKWÄSSER—II

D. TOMIĆ

Pharmakologisches Institut der Universität, Salata 11, Zagreb, Jugoslawien

Zusammenfassung—Es wurden zahlreiche Trinkwässer aus Montenegro auf ihren Mineralgehalt untersucht (Tabelle 1, 2). Die Gesamthärte betrug 2,7 bis 15,3° dH. Meistens handelt es sich um weiche und mittelharte Wässer. Der Chloridgehalt bewegt sich zwischen 1,0 mg/l und 11,6 mg/l. Der Fluoridgehalt beträgt 0,035 mg/l bis 0,190 mg/l. Eine Ausnahme macht das Trinkwasser von Risan, welches infiltriertes Meerwasser enthält und einen Halogengehalt von 565,0 mg/l Cl und 0,200 mg/l F aufweist. Der Kariesbefall ist bei Kindern des gebirgigen Binnenlandes viel kleiner (8,2 bis 12,2 Prozent kariöser Zähne) als bei Kindern des Küstenlandes (22,1 bis 68,2 Prozent kariöser Zähne). Der Mangel des kariesverhütenden Einflusses des Fluorid- bzw. Mineralgehaltes des Trinkwassers wird in Montenegro von anderen Ernährungsfaktoren verdeckt.

Abstract—A large number of drinking waters were examined for their mineral content in Montenegro (Tables 1, 2). The total hardness was 2·7–15·3° dH. The majority of the waters are soft or medium hard. The chloride rate lies between 1 and 11·6 mg/l. The fluoride content lies between 0·035 and 0·190 mg/l. The drinking water of Risan offers an exception; it contains a certain amount of sea water and shows halogens at 565·0 mg/l Cl. and 0·200 mg/l F.

Children of the mountainous inland areas show far less caries than the children of the coast, namely 8·2–12·2 per cent and 22·1–68·2 per cent carious teeth respectively.

The lack of caries-preventing influences of fluorine and minerals in drinking waters of Montenegro is covered up by other factors of nutrition.

DIE ZAHNKARIES kann man zu den Mangelkrankheiten zählen, an deren Entstehung einige Faktoren beteiligt sind. Unter anderem hat der Fluoridgehalt der Nahrung bzw. des Trinkwassers besondere Bedeutung.

In diesem Zusammenhang untersuchten wir zahlreiche Trinkwässer aus Montenegro (im Mai, Juni 1950 TOMIĆ, 1951 und Juli, November 1960), wobei wir nicht nur den Fluoridgehalt, sondern auch die Härte und den Chloridgehalt (HAASE, 1954) der Wässer bestimmten, da der Kalzium- und Magnesiumgehalt ebenfalls für die Entstehung der Zahnkaries Bedeutung hat. Ein hoher Chloridgehalt des Trinkwassers lässt in Küstengebieten auf eine Beimischung von Meerwasser schliessen.

Montenegro (Crna Gora), eine der Volksrepubliken Jugoslawiens, umfasst ein Gebiet von 13 967 km² mit etwa 420 000 Einwohnern*. Dieses schwach besiedelte

* *Enciklopedija Jugoslavije*, Bd. 2, S. 398, Zagreb (1956).

Land (30 Einwohner/km²) stellt keine geographische Einheit dar. Es wird grösstenteils von den faltenreichen Dinarischen Alpen oder Karst eingenommen, deren Hochflächen Trias- und Kreidekalk mit Sandstein und Schiefereinlagen bilden. Im nordöstlichen Teil des Landes treten paläozoische Schiefer und Sandsteine zu Tage, oft von porphyrischen Eruptivgesteinen durchbrochen. Im Tiefland, nördlich vom Skutarisee, längs der Morača und unteren Zeta finden sich Quartärablage-

ABB. 1.

rungen. Zwischen Hercegnovi und Budva, sowie bei Ulcinj bilden Tertiärgesteine einen schmalen Küstensaum, indem nördlich von Stari Bar neben Schiefer auch basisches Eruptivgestein vorkommt.

Die Niederschlagsmenge beträgt im Norden des Landes etwa 700–800 mm im Jahre, sie nimmt gegen den Süden ständig zu und erreicht im gebirgigen Hinter-

land der Küste ein Maximum von über 4000 mm. Die Küste und ihre Umgebung hat ein typisch mediterranisches Wasserregime. In Cetinje beträgt die Regenmenge 3500 mm im Jahre, wobei in den heissen 3 Sommermonaten weniger als 50 mm Regen fällt. Trotz der hohen Niederschlagsmenge zählt der wasserdurchlässige Karstboden zu den wasserärmsten Gebieten und wird fast nur als Weide benützt. Soweit keine besonderen Wasserleitungen vorhanden sind, benützt die spärliche Bevölkerung zu Genusszwecken Regenwasser, welches von den Dächern der Wohngebäude oder auf betonierten Auffangflächen gesammelt und in besondere Behälter (Zisternen) geleitet wird. So z. B. in Velimlje. Orte die sich auf wasserundurchlässigen Schichten befinden, haben oft Brunnen zur Trinkwasserversorgung (Ivangrad, Titograd, Žabljak). Der Norden des Landes wird von der Tara, Piva und dem Lim über die Drina in tief eingeschnittenen Tälern zur Donau entwässert. Im Süden leiten die Morača, Zeta und Rijeka ihr Wasser über den Skutarisee zur Adria. Im Hochgebirge findet man einige Moränenseen (Crno jezero, Plavsko jezero). (Abb. 1).

Vol. 6
1961

DAS MONTENEGRINISCHE BINNENLAND

Im Inneren Montenegros dient als Trinkwasser hauptsächlich Grundwasser, welches in seiner Zusammensetzung ziemliche Unterschiede aufweist (Tabelle 1). Der Abdampfrückstand beträgt hier 83 mg/l (Bijelo Polje) bis 265 mg/l (Grahovo).

TABELLE 1. TRINKWÄSSER DES MONTENEGRINISCHEN BINNENLANDES

Ort und Untersuchungsjahr	Abdampf-rückstand 105°C (mg/l)	Alkalität Karbonat-härte (°dH)	Gesamt-härte (°dH)	Chlorid-gehalt (mg/l)	Fluorid-gehalt (mg/l)
Bijelo Polje, Leitungswasser (1950)	83	2,2	2,7	5,3	0,120
Cetinje, Leitungwasser (1950)	152	7,1	7,3	3,9	0,050
Danilov Grad, Leitungswasser (1950)	136	6,4	6,9	4,8	0,055
Gradac, Leitungswasser (1960)	221	10,4	10,6	2,5	0,080
Grahovo, Leitungswasser (1960)	265	14,0	14,3	3,5	0,060
Ivangrad (Berane), Brunnen, Ul. Mitrovića (1960)	188	8,0	8,1	3,0	0,050
Kolašin, Leitungswasser (1950)	102	4,8	5,2	1,7	0,055
Mojkovac, Quellwasser, Ortsmitte (1960)	180	6,1	7,8	7,5	0,060
Nikšić, Leitungswasser (1950)	122	6,0	6,3	2,9	0,055
Plav, Quellwasser, Ortsmitte (1960)	193	6,7	7,5	1,0	0,190
Pljevlja, Leitungswasser (1950)	217	10,7	11,0	2,6	0,035

(Tabelle 1.-*Fortsetzung*)

Ort und Untersuchungsjahr	Abdampfrückstand 105°C (mg/l)	Alkalität Karbonathärte (°dH)	Gesamthärte (°dH)	Chloridgehalt (mg/l)	Fluoridgehalt (mg/l)
Rožaj, Leitungswasser (1960)	250	11,8	12,3	1,0	0,050
Šavnik, Quellwasser, Ortsmitte (1960)	190	9,3	9,5	2,8	0,070
Titograd (Podgorica), Brunnen bei der Post (1950)	217	8,5	10,0	9,4	0,080
Tomaševo (Šahovići), Quellwasser (1960)	249	8,4	8,7	10,0	0,140
Velimlje, Zisternenwasser (1950)	74	2,7	2,9	3,8	0,050
Vir, Leitungswasser (1960)	220	11,0	12,6	5,5	0,050
Žabljak, Brunnen bei der Post (1950)	367	12,8	15,3	11,6	0,070

Nur das Trinkwasser von Žabljak unterm Durmitor, in dessen westlicher Umgebung Eruptivgestein zu Tage tritt, hat einen etwas grösseren Abdampfrückstand (367 mg/l). Die Härte des Wassers besteht im wesentlichen aus Kalzium- und Magnesiumhydrokarbonat; daher ist der Unterschied zwischen der Karbonathärte (2,2 bis 14,0°dH) und Gesamthärte (2,7 bis 15,3°dH) der einzelnen Wässer im allgemeinen gering. Das Trinkwasser von Bijelo Polje und Velimlje ist nach den gebräuchlichen Härtestufen sehr weich. Cetinje, Danilov Grad, Kolašin, Mojkovac, Nikšić und Plav haben weiches Trinkwasser. Mittelhartes Wasser haben Gradac, Ivangrad (Berane), Pljevlja, Šavnik, Titograd (Podgorica) und Tomaševo (Šahovići). Etwas hart ist das Trinkwasser in Grahovo, Rožaj, Vir (Virpazar) und Žabljak unterm Durmitor. Diese Orte befinden sich in Triasschichten. Der Chloridgehalt beträgt 1,0 mg/l (Plav) bis 11,6 mg/l (Žabljak). Der Fluoridgehalt ist gering; er beträgt im Mittel etwa 0,070 mg/l. Den kleinsten Fluoridgehalt hat Pljevlja (0,035 mg/l), den grössten Plav (0,190 mg/l).

DAS MONTENEGRINISCHE KÜSTENLAND

Die Trinkwässer des Küstenlandes haben eine ähnliche Beschaffenheit wie die Wässer des Binnenlandes (Tabelle 2). Der Abdampfrückstand schwankt zwischen 144 mg/l (Ulcinj) und 186 mg/l (Budva). Es sind dies weiche (Kotor (Cattaro), Ulcinj) und mittelharte Wässer (Budva, Hercegnovi, Stari Bar) mit einer Karbonathärte von 6,4 bis 8,6°dH bzw. Gesamthärte von 6,7 bis 9,0°dH. Der Chloridgehalt beträgt hier 6,2 mg/l (Kotor) bis 10,2 mg/l (Ulcinj). Der Fluoridgehalt ist auch im Küstenland gering und bewegt sich zwischen 0,070 mg/l (Kotor) und 0,100 mg/l (Stari Bar). Eine Ausnahme macht nur das Trinkwasser von Risan. Der sehr grosse

TABELLE 2. TRINKWÄSSER DES MONTENEGRINISCHEN KÜSTENLANDES

Ort und Untersuchungsjahr	Abdampfrückstand 105°C (mg/l)	Alkalität Karbonathärte (°dH)	Gesamthärte (°dH)	Chloridgehalt (mg/l)	Fluoridgehalt (mg/l)
Budva, Leitungswasser (1950)	186	8,5	9,0	10,0	0,075
Hercegnovi, Leitungswasser (1950)	179	8,6	8,9	8,2	0,075
Kotor (Cattaro), Leitungswasser (1950)	153	7,1	7,4	6,2	0,070
Risan, Quellwasser, Krankenhaus (1960)	1318	7,3	17,5	565,0	0,200
Stari Bar, Leitungswasser (1960)	170	7,7	8,3	9,0	0,100
Ulcinj, Leitungswasser (1950)	144	6,4	6,7	10,2	0,085

Abdampfrückstand (1318 mg/l) und Chloridgehalt (565,0 mg/l) deuten auf eine Beimischung von Meerwasser, welches das Quellgebiet infiltriert. Dementsprechend sind auch die Gesamthärte (17,5°dH) und der Fluoridgehalt (0,200 mg/l) mässig erhöht.

DIE ZAHNKARIESHÄUFIGKEIT

RITTERMAN und ERAK (1952) untersuchten die Häufigkeit der Zahnkaries im Inneren und längs der Küste von Montenegro. Sie kamen zu dem Ergebnis, dass die Zahnkariesfrequenz auf den schwerzugänglichen Hochflächen im Inneren des Landes am geringsten ist. So haben Kinder im Durchschnittsalter von 9½ Jahren in Šavnik (1400 m Seehöhe) 12,2 Prozent kariöse Zähne, in Žabljak (1500 m Seehöhe) im Alter von 14¼ Jahren 8,2 Prozent kariöse Zähne. Der Kariesbefall nimmt in der Richtung zur Meeresküste zu und beträgt bei Kindern von 9 Jahren in Budva 68,2 Prozent, in Stari Bar bei 13 jährigen 22,1 Prozent. Ähnliche Ergebnisse zeigten auch die Untersuchungen im Kroatischen Küstenland (TOMIĆ, RITTERMAN und UDOVIĆ-ERAK, 1949).

Den grossen Unterschied in der Zahnkariesfrequenz bei den Bewohnern des gebirgigen Binnenlandes und den Küstenbewohnern kann man mit der verschiedenen Ernährungsweise erklären. Der Fluoridgehalt des Trinkwassers ist sowohl an der Küste, als auch im Inneren von Montenegro sehr gering und kommt daher als kariesverhütender Faktor ebenso wie in Dalmatien (TOMIĆ, 1960) nicht zur Geltung. Ausschlaggebend ist die primitive Ernährungsweise in den vom Verkehr abgeschlossenen Gebirgsgegenden, wo die grösstenteils aus Hirten bestehende Bevölkerung selbstbereitetes Schwarzbrot und viel Milchprodukte geniesst.

LITERATUR

Haase, L. W. 1954. *Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung*, S. 38, 106, 115. Weinheim/Bergstr.

Ritterman, V. und Erak, R. 1952. Frekvencija zubnog karijesa u visinama i Primorskim krajevima Crne Gore (The frequency of dental caries in the mountain and coastal areas of Montenegro) *Liječnički Vjesnik* **74**, 159.

Tomić, D. 1951. Istraživanja voda N.R. Crne Gore s osobitim obzirom na koncentraciju fluora (Untersuchung von Wässern aus Montenegro mit besonderer Berücksichtigung des Fluorgehaltes). *Acta Pharm. Jug.* **1**, 47.

Tomić, D. 1960. Der Fluoridgehalt dalmatinischer Trinkwässer. *Ges.-Ing.* **81**, 24.

Tomić, D., Ritterman, V. Udović-Erak, R. 1949. Odnos zubnog karijesa prema sadržaju fluora u vodi za piće Hrvatskog Primorja (Relation of dental caries to fluorine in the drinking water in Croatian littoral). *Folia stomatol.* **10**, 85.

Vol.
196

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 209–213, 1961. Pergamon Press Ltd.

AN EPIDEMIOLOGIC STUDY OF THE CARIES INCIDENCE IN LOCALITIES WITH AN OPTIMAL CONTENT OF FLUORINE IN DRINKING WATER

M. JIRÁSKOVÁ

Institute for Dental Research, Stalinova 48, Prague 12, Czechoslovakia

Vol. 6
1961

Abstract—In the Czechoslovak Socialist Republic there are localities with a natural higher content of fluorine in drinking water amounting to 1–5 mg F/l. In a locality with an optimal content of fluorine, repeated examinations have been carried out concerning the caries incidence and the condition of the gingiva in children, and the caries incidence in adults.

The reduction of the caries incidence in children was 80 per cent in the permanent dentition as compared with a control group. In addition the eruption rate of the permanent teeth was followed up. It has been found that the replacement of the deciduous by the permanent teeth was retarded in comparison with the average value in the whole state. The author is of the opinion that this is due to the reduction of caries and the reduction of prematurely lost teeth, because the first permanent molars, having no precursors, erupt in time. The incidence of gingivitis is in the fluorine area significantly lower.

The average number of extractions in adults is lower by approximately 40 per cent. The caries in adults is reduced by about one quarter as compared with the control group. This finding is in agreement with the observation in children, because both the reduction of caries in children by 80 per cent and the reduction of caries in adults by 25 per cent requires a reduction of the susceptibility to caries of single teeth by about 75 per cent.

Zusammenfassung—In der C.S.R. gibt es Orte mit einem natürlichen, höheren Fluorgehalt des Trinkwassers von etwa 1–5 mg F/l. In einem Ort mit optimalem Fluorgehalt wurden wiederholt Untersuchungen angestellt, die sich mit dem Kariesvorkommen und dem Zustand der Gingiva von Kindern und der Karieshäufigkeit bei Erwachsenen beschäftigen.

Die Verminderung der Karies bei Kindern betrug an den bleibenden Zähnen 80 Prozent der Kontrollgruppe. Außerdem wurde die Zahnungsgeschwindigkeit der bleibenden Zähne beobachtet. Es zeigte sich, daß die Verdrängung der Milchzähne durch die bleibenden Zähne im Vergleich zum Landesdurchschnitt verzögert auftrat. Die Autorin meint, daß dies auf den geringeren Kariesbefall und auf eine Verminderung der vorzeitig verlorenen Zähne zurückzuführen ist, weil die ersten bleibenden Molaren, die keine Vorläufer haben, in der üblichen Zeit durchbrechen. Das Vorkommen von Gingivitis ist in den Fluorgebieten wesentlich geringer. Die durchschnittliche Zahl der Extraktionen bei Erwachsenen ist ungefähr um 40 Prozent niedriger. Die Karies wird bei Erwachsenen etwa um 1/4 der Kontrollgruppe gesenkt. Dieser Befund steht in Übereinstimmung mit den Beobachtungen

an Kindern, weil beides, die Verminderung der Karies bei Kindern um 80 Prozent und die Verminderung der Karies bei Erwachsenen um 25 Prozent, die Verminderung der Kariesanfälligkeit eines einzelnen Zahnes um 75 Prozent erfordert.

IN CZECHOSLOVAKIA there are numerous places where the drinking water contains 1–5 mg of fluorine per litre. We have carried out repeated teeth examinations in a certain area where the drinking water contains the optimum fluorine content as we consider it a model example of the situation which, under our conditions, we are endeavouring to achieve by fluoridation of water.

In this locality we carried out stomatological examinations of school children as well as adults of 20–50 years of age. In the case of children we observed the occurrence of caries, the eruption of permanent teeth and periodontal diseases.

The reduction of caries of permanent teeth in children of 6–14 years of age, amounted to 80 per cent, in comparison with the control group.

Observation of the shedding of deciduous teeth for permanent ones showed extremely interesting results. We evaluated the eruption of permanent teeth in this group according to the average number of erupted teeth in each child of the same age.

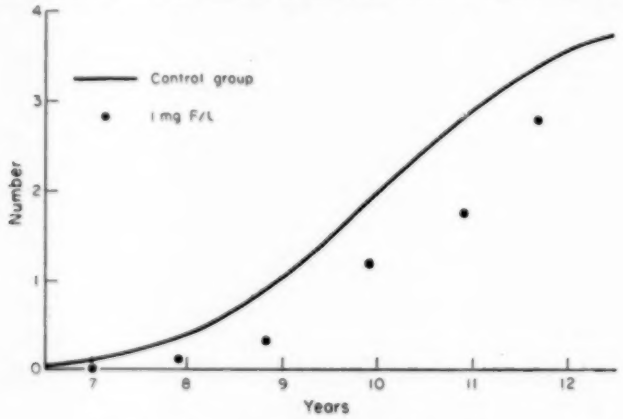


FIG. 1. Average number of erupted premolars.

In comparison with the general average, which we obtained by carrying out random examinations of persons in the individual age groups all over the country, in fluorine localities the time which elapsed between exfoliation of the deciduous molars and eruption of the premolars was shorter, eruption of the permanent teeth replacing the first teeth being delayed. The decrease of the period between the exfoliation of the lower deciduous molars and the eruption of the premolars from 15 months to 11 months, namely a reduction of 4 months, is highly significant. Equally significant was the delayed eruption of the permanent front teeth. This delay was not, of course, the same in the case of all groups of teeth, being the most conspicuous in the premolars (Fig. 1).

We are of the opinion that these differences can be explained solely by reduction of the caries of deciduous teeth. As a consequence of the high occurrence of caries of the deciduous teeth in non-fluoridated areas, these teeth are lost somewhat prematurely, the eruption of those following them therefore being accelerated. The correctness of this opinion is testified by the fact that the period of eruption of the first permanent molars in fluoridated areas is in a full accordance with that observed in the control group. Thus it can be concluded that in fluoridated regions the time of eruption is physiological.

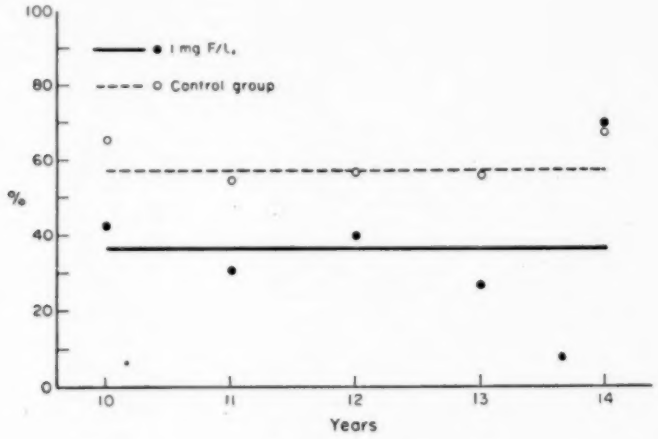


FIG. 2. Percentage of periodontal diseases in boys.

Apart from the effect of fluorine on the teeth we also studied its influence on the gums in children of 10–14 years of age. Younger children were not examined. In order to eliminate periodontal diseases caused by decayed deciduous teeth children with approximal fillings were also not examined since such fillings could be the external cause of periodontal diseases. Inflammation was diagnosed in such cases in which compression of the ridge of the explorer on the interdential papile caused bleeding. In the fluoridated areas the appearance of periodontal diseases proved to be significantly lower than in the control group (Fig. 2). Moreover, its extent (Fig. 3), evaluated according to the number of teeth in the case of which inflammation of the gum was diagnosed, was outstandingly lower than in the control group.

With regard to the cariogenity of adults, this was approximately one quarter lower (about five teeth) in men and women compared with the control group. The average number of extracted teeth was about 40 per cent lower. These average values were calculated in respect of the age of 35 years, due regard being paid to the relation of cariogenity to age. For our control group we choose inhabitants

of localities in the nearest vicinity but whose drinking water contained no fluorine, (Fig. 4).

The average reduction of DMF, amount in adults to about 25 per cent, seems, in comparison with that ascertained in children, namely 80 per cent, relatively

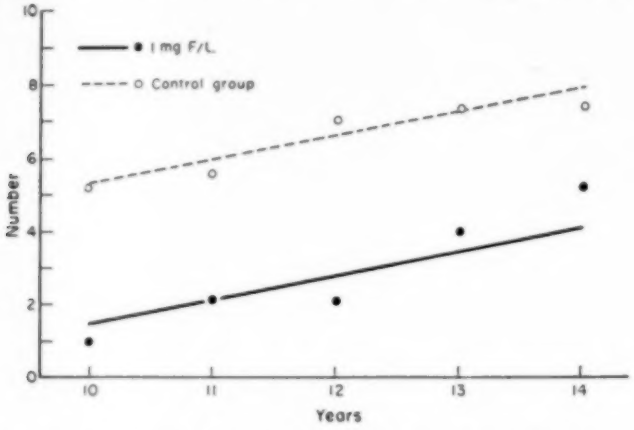


FIG. 3. Average extent of periodontal diseases in boys (Evaluated according to number of periodontally diseased teeth).

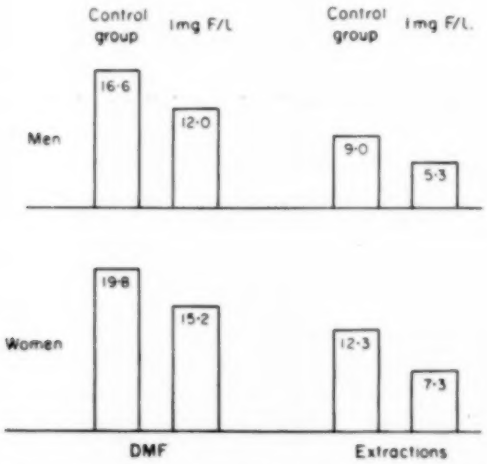


FIG. 4. Average number per one inhabitant (average age 35 years).

low. Nevertheless, this difference corresponds on the whole to the reduction or caries ascertained in children, being, however, caused by the fact that the number of DMF in regions where there is no fluorine in the drinking water increases only very slowly with advancing age (when only relatively resistant teeth are still sound).

On the other hand, in fluoridated regions this effect is manifested in a far smaller extent since a considerably greater number of teeth remain sound. In the mouth there are on the average about five teeth more, while the number of teeth which can be treated is considerably much higher.

It is for this reason that in fluoridated areas we encounter small prosthetic appliances at a much higher age than is usually the case. From this it results that the periodontium is far more protected against overloading and other changes caused by prosthetic works. Here the natural masticatory function of the teeth is preserved much longer.

These facts are very instructive and encouraging since they have proved beyond all doubt the clearly favourable effect of fluorine up to adult age.

Vol. 6
1961

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 214–220, 1961. Pergamon Press Ltd.

EFFECTS OF MASTICATION AND PARTICLE SIZE OF CORN AND SUGAR DIETS ON CARIES-INCIDENCE IN RATS

K. G. KÖNIG

Experimental Caries Research Laboratory, Dental Institute, University of Zurich, Switzerland

Abstract—Caries-incidence in lower jaws of 108 Osborne-Mendel rats (with unilaterally extracted upper molars), given different diets with equal contents of carbohydrate for 20 days, was studied on serial undecalcified sections. The cariogenicity of all combinations of readily fermentable coarse and fine sugar and less readily fermentable coarse and fine corn was investigated in a 3^2 factorial experiment including these nine treatments:

fine sugar	7	8	9
coarse sugar	4	5	6
no sugar	1	2	3
	no corn	coarse corn	fine corn

The basic content of diets 2–9 was 66 per cent corn or sugar, 32 per cent skim milk powder, 2 per cent dried liver.

The analysis of lesion totals on functional plus non functional lower molars indicated: (1) corn is less cariogenic than sugar; (2) coarse sugar is less cariogenic than fine sugar; (3) coarse corn is less cariogenic than fine corn; (4) supplementation of half of the sugar by corn reduced the cariogenicity of sugar.

Diets containing corn only (2,3) caused less lesions on the nonfunctional than on the functional side, whereas diets containing sugar only (4,7) caused more lesions on the nonfunctional than on the functional side. Diet 7 (= diet Stephan 580) was found to be the most cariogenic of all combinations tested.

Zusammenfassung—Der kariöse Befall der Unterkiefermolaren von 108 Albinoratten, denen rechtsseitig die Oberkiefermolaren extrahiert worden waren, wurde nach 20tägiger Verfütterung verschiedener Diäten mit konstantem Kohlehydratgehalt (66 Prozent) mittels der Serienschliffmethode untersucht. Die Kariogenizität der Diätkombinationen mit (leicht fermentierbarem) grobem und feinem Zucker und (schwer fermentierbarem) grobem und feinem Mais wurde in einem 3^2-Faktorenversuch mit folgenden 9 Verfahren geprüft:

Feiner Zucker	7	8	9
Grober Zucker	4	5	6
Kein Zucker	1	2	3
	Kein Mais	Grober Mais	Feiner Mais

Im Prinzip enthielten die Diäten 66 Prozent Mais oder Zucker, 32 Prozent Magermilchpulver und 2 Prozent Leber. Die Analyse der Gesamtbefallszahlen rechts + links zeigte: (1) Mais ist weniger kariogen als Zucker; (2) grober Zucker ist weniger kariogen als feiner Zucker; (3) Grober Mais ist weniger kariogen als feiner Mais; (4) Teilersatz von Zucker durch Mais verringert die Kariogenizität des Zuckers, was nicht

allein auf die Reduktion des absoluten Zuckergehaltes zurückgeführt werden kann. Vergleiche des Befalls zwischen rechts und links zeigen bei Mais als alleinigem Kohlehydrat (2,3) weniger Läsionen auf der afunktionellen, bei Zucker allein (4,7) mehr Läsionen auf der afunktionellen als auf der funktionellen Seite. Die Diät in Verfahren 7 (= Stephan 580; 66 Prozent feiner Zucker, 32 Prozent Milchpulver, 2 Prozent Leber) war von allen geprüften Kombinationen am stärksten kariogen.

Vol. 6
1961

INTRODUCTION

EARLY WORK on the production of experimental dental caries in laboratory rats seemed to indicate that coarse particles of corn or rice would be necessary to induce or at least to initiate, carious lesions which did not occur in general when finely ground corn or rice were used (HOPPERT, WEBBER and CANNIFF, 1932; KLEIN and MCCOLLUM, 1933; ROSEBURY, KARSHAN and FOLEY, 1933a, 1934; GINN, 1942; HUNT, HOPPERT and ERWIN, 1944; COX, 1948; NAKFOOR, HUNT and HOPPERT, 1952). The question arose whether the cusp fractures caused by coarse particles, complicating evaluation, were preceded or followed by the development of carious lesions (MCCOLLUM *et al.*, 1922; ROSEBURY, KARSHAN and FOLEY, 1933b; SHELLING and ASHER, 1933; KEARNY, SOGNNAES and BIBBY, 1941; VAN HUYSEN and VINCENT, 1945; VAN HUYSEN, 1950). As has been shown by studies on the effect of mastication (SOGNNAES, 1941a, b), impaction into the fissures is necessary to retain the corn particles long enough to secure utilization by mouth bacteria of these slowly fermentable carbohydrates. Smooth surface lesions, common in human teeth, were not observed after feeding coarse particle diets, and there were failures in early attempts to produce experimental dental caries on high-sucrose diets (LILLY, 1932; LILLY and GRACE, 1932; MARSHALL-DAY, DAGGS and SEDWICK, 1935). In these early days, there were reasons enough to question the identity of the aetiology of experimental rat caries with the basic aetiology of human dental caries (ROSEBURY and FOLEY, 1932; BODECKER and APPLEBAUM, 1932; MCCLURE, 1948, 1955).

When, in 1942, ARNOLD for the first time used hamsters in caries experiments, he in some respect obtained more reasonable results than had been attained up to this time in rats. He found a diet containing 66 per cent finely ground corn more cariogenic than the same diet containing 66 per cent coarse corn. Moreover, ARNOLD observed cervical smooth surface lesions. These findings led to the conclusion that the hamster was a laboratory animal better suited for caries experiments than the rat.

Since then the production of uniformly susceptible inbred strains of rats, better knowledge of the effects of different constituents of experimental diets, and increasing experience in running studies of more complicated design have been achieved. These advancements have made it possible to reevaluate the influence of coarse and fine corn and sugar with and without masticatory stress, using short experimental periods, microscopic evaluation of undecalcified serial sections, and the rat as an experimental animal.

MATERIAL AND METHODS

In 108 weanling Osborne-Mendel rats (twelve litters of nine) the right upper first and second molars were extracted under anaesthesia. 2 days later the animals were littermate distributed over the following nine feeding regimens in a kind of a 3^2-factorial design, each regimen being tested on twelve animals:

fine sugar*	7	8	9
coarse sugar*	4	5	6
no sugar	1	2	3
	no corn	coarse corn*	fine corn*

With the exception of Diet 1 all diets contained 66 per cent corn or sugar, 32 per cent skim milk powder, and 2 per cent whole dried liver. Diets 4 and 7 contained sugar only (66 per cent) Diets 2 and 3 corn only (66 per cent), the diets with both carbohydrates, 5, 6, 8 and 9, contained 33 per cent corn and 33 per cent sugar. Diet 1 without sugar and corn consisted of 96 per cent skim milk and 4 per cent liver. Weight gains were controlled every 5 days.

After an experimental period of 20 days feeding the nine different diets, the animals were sacrificed and the jaws removed and fixed in 10% formalin. The lower jaws were inspected for cervical smooth surface lesions and scores assigned according to KEYES (1958). Then undecalcified serial sagittal sections were prepared with the JANSEN (1950) circular saw, stained with Schiff's reagent and examined microscopically for sulcal lesions (KÖNIG, MARTHALER and MÜHLEMANN, 1958). The lesions were scored as follows (with corresponding KEYES' (1958) nomenclature in parentheses):

A (E) = enamel lesion;

T (Ds) = involvement of dentine–enamel junction;

B (Dm) = moderate dentinal lesion;

C (Dx) = extensive dentinal lesion with undermining of cusps and beginning cavitation discernible macroscopically.

The presence or absence of occlusal attritions was stated by visual comparison of the appearance of cusps on the right and lefts sides and scored as "not present" = −, "doubtful" = (+), "moderate" = +, and "marked" = ++.

All scoring was done under code without knowledge of the regimen to which an animal had belonged.

* Coarse corn: 48 per cent of particles retained by 20-mesh sieve, coarse sugar: 80 per cent of particles retained by 20-mesh sieve, fine corn and sugar: nearly all particles passed 60-mesh sieve.

Vol. 196

RESULTS

Growth

There were marked differences of average weight gains between the regimens. Growth was normal on the corn diets and subnormal on the diets containing no corn. Especially the animals on coarse sugar (Diet 4) failed to gain weight normally.

Caries scores

The average numbers of sulcal dentinal lesions (score B plus more severe ones, total of right + left lower molars, and separately for the right, i.e. non functional, and left, i.e. functional side) and average numbers of cervical lesions (score E plus more severe ones, total of right + left, and separately right and left) are presented in Table 1.

TABLE 1

	*r+l**		*r+l*		*r+l*		Av. No. of lesions
	r	*l*	*r*	*l*	*r*	*l*	
Fine sugar	(7) 9,4		(8) 6,0		(9) 8,6		Sulcal
	5,2	4,2	3,2	2,8	4,3	4,3	
	9,4		2,5		4,8		Cervical
	5,5	3,9	2,2	0,3	2,3	2,5	
Coarse sugar	(4) 5,7		(5) 4,8		(6) 6,6		Sulcal
	3,7	2,0	2,7	2,1	3,9	2,7	
	3,4		3,1		4,3		Cervical
	2,3	1,1	1,7	1,4	2,3	2,0	
No sugar	(1) 0,5		(2) 1,8		(3) 3,2		Sulcal
	0,5	0,0	0,1	1,7	1,2	2,0	
	1,2		0,4		2,2		Cervical
	1,2	0,0	0,4	0,0	1,6	0,6	
	No corn		Coarse corn		Fine corn		

* *r*=right, *l*=left; treatment numbers () in upper left corners.

Cariogenicity of diets containing corn only or sugar only

The total numbers (in functional plus non-functional lower molars) of sulcal and cervical smooth surface lesions in the treatments with only one carbohydrate (Diet 2 with 66 per cent coarse corn, Diet 3 with 66 per cent fine corn, Diet 4 with 66 per cent coarse sugar, Diet 7 with 66 per cent fine sugar) show that corn is less cariogenic than sugar, and that the diets with coarse particles (2 and 4) are less cariogenic than the corresponding diets with fine particles (3 and 7).

The numbers of sulcal lesions evaluated separately for the left, i.e. functional, and the right, i.e. non functional, lower molars indicate the importance of impaction

for the cariogenic properties of the coarse corn (Diet 2), nearly all lesions being located on the functional side. On the fine corn diet (3) the increase of caries as compared to the coarse corn is mainly due to lesions occurring in the non-functional molars, the fine corn being sticky and adhesive enough to be retained without impaction by masticatory stress. By contrast to the corn diets, on the coarse and fine sugar diets more sulcal lesions occurred in the non functional teeth, the difference between function and non-function being greater in the mechanically active coarse sugar diet (4).

The number of cervical smooth surface lesions was greater in the non-functional molars on all regimens, indicating the aetiologic importance of lacking function and plaque formation.

Cariogenicity of diets containing corn and sugar

Compared with the effect of diets containing sugar as the only carbohydrate, substitution of half of the coarse or fine sugar by coarse corn reduced the number of sulcal lesions (Diets 4 vs. 5 and 7 vs. 8). Cervical caries was reduced only when half of the fine sugar was substituted by coarse corn (Diet 7 vs. 8), the substitution of coarse corn apparently not adding very much to the selfcleaning effect of the 66 per cent coarse sugar diet (5 vs. 4).

Substitution of half of the sugar by finely ground corn (Diets 4 vs. 6 and 7 vs. 9) did not significantly reduce the number of sulcal lesions in comparison to the incidence on diets containing sugar only. On the contrary, in spite of the high cariogenicity of pure sugar diets, 33 per cent coarse sugar and 33 per cent fine corn (Diet 6) were more cariogenic than 66 per cent coarse sugar (Diet 4).

Attrition of cusps in functioning molars

Marked occlusal attrition of cusps was found in most of the animals on the three diets containing coarse sugar (4, 5 and 6). The figures of "no" plus "doubtful" and of "moderate" plus "marked" attritions observed in the different treatments are given in Table 2.

TABLE 2

				Attrition
Fine sugar	9	10	9	—, (+)
	3	2	3	+, ++
Coarse sugar	3	0	0	—, (+)
	9	12	12	+, ++
No sugar	8	9	12	—, (+)
	4	3	0	+, ++
	No corn	Coarse corn	Fine corn	

Comparing within the three times three groups of diets

firstly, without sugar (Diets 1, 2 and 3)

secondly, with coarse sugar (Diets 4, 5 and 6)

thirdly, with fine sugar (Diets 7, 8 and 9),

no significant abrasive effect was observed which could be attributed to the mastication of coarse corn.

DISCUSSION

The early findings indicating that corn is cariogenic only in the form of coarse particles (authors cited in the introduction), was not confirmed in the present investigation. However, the results presented are in accordance with the observations of ARNOLD (1942), who found fine corn more cariogenic than coarse corn.

The observation in the present experiment that coarse sugar is less cariogenic than fine sugar is in keeping with the results of STEWART, HOPPERT and HUNT (1953), who found lesions in live rats on powdered sugar after a considerably shorter experimental period than in rats on a granulated sugar diet. The decided abrasive effect of granulated sugar has formerly been described by MCCLURE (1945). In his experiment fine sugar was more cariogenic than coarse sugar, but he had used highly resistant, rats and failed to reproduce carious lesions at all on excessive quantities of dietary sugar in subsequent experiments.

The validity of the present study is confirmed by the identical results of the first (seven rats in each regimen) and of the second part (five rats in each regimen) of the experiment, the second being run 3 months later than the first.

REFERENCES

ARNOLD, F. A., Jr. 1942. The production of carious lesions in the molar teeth of hamsters (*C. auratus*). *Publ. Health Rep.*, Wash. **57**, 1599–1604.

BÖDECKER, C. F. and APPLEBAUM, E. 1932. Experimental caries in rats. *Dent. Cosmos* **74** 335–339.

COX, G. J., DODDS, M. L., LEVIN, M. M. and HODGE, H. C. 1948. The effect of carbohydrates on experimental caries in the rat. *J. dent. Res.* **27**, 562–575.

GINN, J. T. 1942. Importance of mechanical factors upon the progress of experimental dental caries in the rat. *J. dent. Res.* **21**, 333.

HOPPERT, C. A., WEBBER, P. A. and CANNIFF, T. L. 1932. The production of dental caries in rats fed an adequate diet. *J. dent. Res.* **12**, 161–173.

HUNT, H. R., HOPPERT, C. A. and ERWIN, W. G. 1944. Inheritance of susceptibility to caries in albino rats (*Mus norvegicus*) *J. dent. Res.* **23**, 385–401.

JANSEN, M. T. 1950. An improved method for the preparation of "serial" sections of undecalcified dental tissues. *J. dent. Res.* **29**, 401–406.

KEARNEY, B., SOGNNAES, R. F. and BIBBY, B. G. 1941. Pathogenesis of coarse corn rat caries. *J. dent. Res.* **20**, 280.

KEYES, P. H. 1958. Dental caries in the molar teeth of rats—II. A method for diagnosing and scoring several types of lesions simultaneously. *J. dent. Res.* **37**, 1088–1098.

KLEIN, H. and MCCOLLUM, E. V. 1933. The significance of food-particle size in the aetiology of macroscopic dental decay in rats. *J. dent. Res.* **13**, 69–71.

KÖNIG, K. G., MARTHALER, T. M. and MÜHLEMANN, H. R. 1958. Methodik der kurzfristig erzeugten Rattenkaries. *Dtsch. Zahn-, Mund-, u. Kieferheilk.* **29**, 99–127.

Vol. 6
1961

LILLY, C. A. 1932. Failures to produce experimental dental caries in the white rat with high carbohydrate diet and bacillus acidophilus or with vitamin D deficiency *J. Nutr.* **5**, 175–181.

LILLY, C. A. and GRACE, D. 1932. Failure to produce dental caries with high carbohydrate, and with extremely low fat diets. *Proc. Soc. exp. Biol., N. Y.* **30**, 176–177.

MARSHALL-DAY, C. D., DAGGS, R. G. and SEDWICK, H. J. 1935. High sugar diets and dental caries in the white rat. *J. Amer. dent. Ass.* **22**, 913–925.

MCCLURE, F. J. 1945. Observations on induced caries in rats—V. Effects of excessive sugar in the diet. *J. dent. Res.* **24**, 239–245.

MCCLURE, F. J. 1948. Observations on induced caries in rats—VI. Summary results of various modifications of food and drinking water *J. dent. Res.* **27**, 34–40.

MCCLURE, F. J. 1955. Dietary factors in experimental rat caries. In: SOGNNAES, R. F. (Ed.): *Advances in Experimental Caries Research.* pp. 107–124. A.A.A.S., Washington.

MCCOLLUM, E. V., SIMMONDS, N., KINNEY, E. M. and GRIEVES, C. J. 1922. The relation of nutrition to tooth development and tooth preservation—I. A preliminary study of gross maxillary and dental defects in two hundred and twenty rats on defective and deficient diets. *John Hopkins Hosp. Bull.* **33**, 202–215.

NAKHOOR, E. C., HUNT, H. R. and HOPPERT, C. A. 1952. Fracturing of the molar teeth in caries-susceptible and caries-resistant albino rats (*Rattus norvegicus*). *J. dent. Res.* **31**, 143–150.

ROSEBURY, T. and FOLEY, G. 1932. Studies, in the rat, of susceptibility to dental caries—II. The effect on mothers and young, of diets deficient in calcium and vitamin D, during pregnancy, lactation, and weaning. *J. dent. Res.* **12**, 462–464.

ROSEBURY, T., KARSHAN, M. and FOLEY, G. 1933a. Studies, in the rat, of susceptibility to dental caries—III. The experimental production of typical dental fissure-caries and other lesions in rats, and preliminary studies of their etiology. *J. dent. Res.* **13**, 379–398.

ROSEBURY, T., KARSHAN, M. and FOLEY, G. 1933b. Production of typical dental caries and other lesions in rats; preliminary study of their etiology. *J. dent. Res.* **13**, 247.

ROSEBURY, T., KARSHAN, M. and FOLEY, G. 1934. Studies, in the rat, of susceptibility to dental caries—IV. Further studies of the etiology of fissure caries. *J. Amer. dent. Ass.* **21**, 1599–1611.

SHELLING, D. H. and ASHER, D. E. 1933. Calcium and phosphorus studies—VIII. Some observations on the incidence of caries-like lesions in the rat. *J. dent. Res.* **13**, 363–378.

SOGNNAES, R. F. 1941a. Studies on masticatory efficiency—II. Masticatory efficiency of rats. *Amer. J. Orthodont.* **27**, 383–388.

SOGNNAES, R. F. 1941b. Studies on masticatory efficiency—IV. Mastication and experimental rat caries. *Amer. J. Orthodont.* **27**, 552–556.

STEWART, W. H., HOPPERT, C. A. and HUNT, H. R. 1953. The incidence of dental caries in caries-susceptible and caries-resistant albino rats (*Rattus norvegicus*) when fed diets containing granulated and powdered sucrose. *J. dent. Res.* **32**, 210–214.

VAN HUYSEN, G. 1950. Microscopical study of caries of rats' teeth. *J. dent. Res.* **29**, 809–814.

VAN HUYSEN, G. and VINCENT, C. J. 1945. Rat caries. *J. dent. Res.* **24**, 203.

Arch. Oral. Biol., Special Supplement, Vol. 6, pp. 221-227, 1961. Pergamon Press Ltd.

ÜBER DIE SPALTUNG VON SACCHAROSE UND MALTOSE IM MENSCHLICHEN SPEICHEL

T. NEUBAUER und R. NAUJOKS

Kieferklinik des Universitätskrankenhauses, Martinistrasse, Hamburg 20, Deutschland

Zusammenfassung—(1) Mit papierchromatographischen Methoden und anschließender qualitativer Bestimmung wird die Spaltung von Saccharose und Maltose im Speichel kariesresistenter und kariesanfälliger Personen gemessen. (2) Die Spaltung von Saccharose erfolgt im Speichel resistenter dreimal und die von Maltose fünfmal so schnell wie im Speichel anfälliger Personen. (3) Die Ursachen für diese Ergebnisse werden diskutiert.

Abstract— (1) By means of paper chromatography and following quantitative determinations, the cleavage of sucrose and maltose in saliva of caries resistant and caries susceptible instances was measured. (2) In saliva of caries resistants the cleavage of sucrose is three times faster and of maltose five times faster than in caries-susceptible individuals. (3) The results are discussed.

IN DER modernen Kariesforschung nehmen die Untersuchungen über den Kohlenhydratabbau in der Mundhöhle einen breiten Raum ein. Das besondere Interesse galt hierbei dem Abbau der Monosaccharide, speziell der Glukose (BRAMSTEDT *et al.*, 1954; BRAMSTED, KRÖNCKE und NAUJOKS, 1956, 1957; LUNDQVIST, 1952; SWENANDER-LANKE, 1957, u.a.). Nur wenige experimentelle Untersuchungen sind dagegen bekannt geworden, die sich mit der Spaltung der Disaccharide im Speichel befassen. Während das Vorhandensein und die Aktivität der Amylase im Speichel gut bekannt sind (FLORESTANO, FASSER and TATES 1941; ERICSSON, 1951; HESS und SMITH, 1948, SULLIVAN und STORVICK, 1950; TURNER und CRANE, 1944, u.v.a.), wurde noch vor kurzem die Auffassung vertreten, daß disaccharidspaltende Fermente in der Mundhöhle nicht existieren. Neuere Untersuchungen konnten jedoch zeigen, daß auch Saccharose, Maltose und Lactose im Speichel abgebaut werden (HARTLES und MCDONALD, 1950; HARTLES und WASDELL, 1954a, b, c; HARTLESS, SLACK und WASDELL, 1954; VOLKER, 1950; SAUERWEIN, 1952; KRÖNCKE, 1959, u.a.).

Darüber hinaus bewiesen die sogenannten "Vipeholm-Untersuchungen" (GUSTAFSON, 1952) eindeutig, daß eine erhöhte Verzehrung von Kohlenhydraten eine gesteigerte Kariesfrequenz hervorruft. Das Vorhandensein von Zuckern und besonders deren Zwischenprodukten in der Mundhöhle über einen längeren Zeitraum wurde hier als einer der Faktoren angesehen, die den Kariesbefall einleiten und die Vergrößerung bzw. Ausbreitung schon vorhandener kariöser Läsionen bewirken können.

Die Verweildauer von Zuckern sowie ihrer Abbauprodukte variiert nach LUNDQVIST (1952), SWENANDER-LANKE (1957) sowie BRAMSTEDT *et al.* (1954, 1956, 1957) auf Grund unterschiedlicher Abbaugeschwindigkeiten in den Mundhöhlen erheblich. Unter Zugrundelegung der Ergebnisse aus diesen Experimenten kann heute kaum noch bezweifelt werden, daß der Abbau der Kohlenhydrate in der Mundhöhle kariesanfälliger Personen langsamer abläuft als in der kariesresistenter. Neben einer verminderten Sauerstoffaufnahme (NAUJOKS und VONDERLINN, 1953; KRÖNCKE und NAUJOKS, 1954) wurden im Speichel kariesanfälliger Personen auch eine signifikant geringere Aldolase- (NAUJOKS *et al.* 1960, 1961) und Hexokinaseaktivität (BRAMSTEDT, NAUJOKS und BENEDIKT, 1961) beobachtet. Bei einem Vergleich der fermentativen Leistungen des Speichels beider Personengruppen wurde ein Verhältnis von 1:3 bis 1:4 festgestellt. Als Substrat diente bei diesen Untersuchungen hauptsächlich Glukose. Die dem menschlichen Organismus in der Nahrung zugeführten Zucker bestehen jedoch im wesentlichen aus Disacchariden wie Rohrzucker oder Malzzucker. Auch die mit der Nahrung zugeführte Stärke wird bekanntlich zu Maltose abgebaut.

Nachdem die vorstehend erwähnten Untersuchungen sichere Hinweise dafür liefern konnten, daß im Speichel kariesanfälliger Personen der Glukoseabbau, im Gegensatz zu kariesresistenten, erheblich verlangsamt abläuft, erschien es von größtem Interesse, festzustellen, ob ähnliche Unterschiede zwischen den beiden Personengruppen auch im Zusammenhang mit der Spaltung von Disacchariden vorliegen.

In den nachstehend beschriebenen Experimenten wird mit Hilfe papierchromatographischer Methoden die Spaltung von Saccharose und Maltose im Speichel untersucht und quantitativ bestimmt.

MATERIAL UND METHODEN

Material

Als Untersuchungsmaterial diente Nüchtern-Ruhespeichel von kariesresistenten und -anfälligen Personen. Die Speichelproben wurden morgens, unmittelbar nach dem Erwachen, entnommen, da nach früher veröffentlichten Untersuchungen (NAUJOKS *et al.*, 1961) nur diese für eine Bestimmung der tatsächlichen Stoffwechselleitungen des Speichels geeignet sind. Die Einteilung der Probanden in kariesanfällige und kariesresistente erfolgte auf Grund des klinischen Zustandsbildes des Gebisses und der Kariesanamnese. Als unterstützende Maßnahme für die Zuordnung der Probanden zu einer der beiden Gruppen wurde die Sauerstoffaufnahme des Speichels herangezogen. Diese hat sich uns (NAUJOKS und VONDERLINN, 1953; KRÖNCKE und NAUJOKS, 1954, zumindest bei Gruppenexperimenten als Kariestest gut bewährt.

Methodik

Inkubation des Speichel-Disaccharidgemisches.

Vol. 6
1961

(a) *Ansatz*

4,3 ml Speichel (s.o.)
2,0 ml Aqua dest.
2,15 ml Phosphatpuffer (pH 7,2).

Im Phosphatpuffer befanden sich außerdem 135 I.E. Hyaloronidase (Luronase-Bayer). Dieser Zusatz erwies sich als notwendig, um die bei der späteren papierchromatographischen Untersuchung sonst störende Viskosität des Speichels herabzusetzen.

Bei der verwendeten Lorinase wurde keine Disaccharidspaltung festgestellt.

(b) *Die Inkubation des Ansatzes erfolgte im Warburgapparat bei* 37°C

1. im Hauptraum des Warburggefäßes befanden sich 2,0 ml des o.a. Gemisches,
2. das Zentralgefäß enthielt 0,2 ml NaOH (7 Prozent),
3. im Seitengefäß befanden sich (9.804 mg Saccharose oder Maltose). 0,3 ml Disaccharidlösung

Nach Zugabe der Zuckerlösung zum Speichel-Puffergemisch entsprach die Disaccharidkonzentration in dem vollständigen Ansatz 1/80 M.

Für die Aufstellung einer Eichkurve wurde der Disaccharidzusatz variiert. Die Zugabe dieser variierten Zuckermengen zum Ansatz erfolgte hier unmittelbar vor der Fermentinaktivierung durch Erhitzen (2 min bei 100°C). Inkubationsdauer bis zu 5 Stunden.

(c) *Papierchromatographische Untersuchungen*

2 ml des Ansatzes wurden nach Inaktivierung mit Aqua dest. auf 11 ml aufgefüllt, Ausfällung der Phosphate mit $MgCl^2$ und NH^2 bei pH 8,8 Zentrifugieren,

0,1 ml des Überstandes absteigend chromotographieren (Aceton: Butanol: Wasser = 7:2:1, mit 2,5% Phtalsäurezusatz), (Papier: Schleicher & Schül Nr. 2043 bMgl (ausgewaschen)).

Papier durch 2-prozentige Anilinlösung in Äther ziehen. Entwickeln 15 Min. bei 130°C.

Anschließend flächengleiches Ausschneiden der entwickelten Flecken und Elution in 10 ml Eisessig für 36 Stunden.

Fotometrische Messung bei 480 mu (5 cm Schichtdicke) gegen einen in gleicher Weise behandelten Blindwert.

Auswertung der Ergebnisse durch graphische Darstellung.

STATISTIK

Die statistische Sicherung der Unterschiede zwischen den Mittelwerten wurde nach dem Signifikanz-*t*-Test nach FISHER (1956) durchgeführt.

Hiernach ist

$$t = (\bar{x}_1 - \bar{x}_2) \left\{ \left[\frac{\sum_1 (x_1 - \bar{x}_1)^2 + \sum_2 (x_2 - \bar{x}_2)^2}{N_1 + N_2 - 2} \right]^{\frac{1}{2}} \left(\frac{1}{N_1} + \frac{1}{N_2} \right) \right\}^{-1}$$

Bei Versuchsreihen von zumindest 8 Einzelversuchen und bei einem Freiheitsgrad von 2 sind t-Werte 3,707 der Beweis für eine mit 99,9 Prozent Wahrscheinlichkeit gesicherte Signifikanz.

ERGEBNISSE

Die Resultate aus unseren Untersuchungen sind in der Tabelle 1 wiedergegeben. Danach werden in 1 ml Speichel pro Stunde bei kariesresistenten 4,65$\pm$0,38 μMol und bei kariesanfälligen Personen 1,45$\pm$0,29 μMol Saccharose im Durchschnitt

TABELLE 1. —SPALTUNG VON SACCHAROSE UND MALTOSE IM NÜCHTERN-RUHESPEICHEL VON KARIESRESISTSTEN (KR) UND KARIESANFÄLLIGEN (KA) PERSONEN (1 ML SPEICHEL/STUNDE)
M = Mittelwerte; t = t-Werte nach dem Signifikanz-t-Test nach FISHER (1956). Die Unterschiede zwischen KR und KA sind mit 99,9 Prozent Wahrscheinlichkeit statistisch gesichert

	Saccharose (μMol)		Maltose (μMol)	
	KR	KA	KR	KA
1.	5,65	1,52	6,07	2,61
2.	2,38	0,45	6,43	2,68
3.	5,65	2,32	7,06	1,27
4.	4,35	0,90	7,06	0,14
5.	4,18	2,09	5,86	1,20
6.	3,00	1,41	4,94	0,35
7.	5,65	1,13	5,44	1,13
8.	5,30	0,28	7,06	0,00
9.	5,65	2,94	6,21	—
10.	—	—	4,24	—
M =	4,65 $\pm$ 0,38	1,45 $\pm$ 0,29	6,04 $\pm$ 0,22	1,17 $\pm$ 0,33
	t = 6,25		t = 10,3	

gespalten. Der Unterschied dieser Mittelwerte ist statistisch signifikant. Der t-Wert beträgt hier (nach dem Signifikanz-t-Test nach FISHER (1956) = 6,25). Die Maltose-Untersuchungen ergaben für 1 ml Speichel pro Stunde bei kariesresistenten Personen 6,04$\pm$0,22 μMol und für kariesanfällige Personen 1,17$\pm$0,33 μMol Maltose. Der Unterschied dieser Durchschnittswerte ist ebenfalls statistisch gesichert. Der t-Wert betrug hier = 10,3. Das Spaltungsvermögen des Speichels für Saccharose zeigt zwischen KA- und KR-Personen ein Verhältnis von ca. 1:3. Für die Maltosespaltung erbrachten die Resultate für die beiden Personengruppen ein Verhältnis von ca. 1:5.

DISKUSSION

Die vorliegenden Untersuchungen konnten die von HARTLES und MCDONALD (1950), HARTLES und WASDELL (1954a, b, c), HARTLES *et al.* (1954), SAUERWEIN (1952), KRÖNCKE (1959), u.a., erzielten Resultate über den Disaccharidumsatz im Speichel einserseits bestätigen, andererseits aber den Beweis dafür erbringen, daß wie schon von KRÖNCKE (1959) angedeutet, zwischen kariesanfälligen und -resistenten Personen bezüglich der Spaltungsgeschwindigkeit von Disacchariden signifikante Unterschiede bestehen.

Nachdem wir schon früher feststellten (BRAMSTEDT *et al.*, 1954, 1956, 1957, 1961; NAUJOKS *et al.*, 1960, 1961), daß die Abbaugeschwindigkeit der Glukose im Speichel der beiden Personengruppen signifikant unterschiedlich ist, haben die vorliegenden Resultate gezeigt, daß auch der Umsatz der Disaccharide im Speichel kariesanfälliger Personen in weit geringerem Umfange erfolgt als in dem kariesresistenter. Neben einer reduzierten Hexokinase- und Aldolaseaktivität (NAUJOKS *et al.*, 1960, 1961; BRAMSTEDT *et al.*, 1961) sowie einer verminderten Sauerstoffaufnahme des Speichels kariesanfälliger Personen dürfte eine verlangsamte Spaltung der Disaccharide ebenfalls ein Characteristikum für die eingeschränkten Stoffwechselleistungen des Speichels dieser Personengruppe sein. Die Ergebnisse sind ein weiterer Hinweis dafür, daß die verlangsamte "Oral sugar clearance" (LUNDQVIST, 1952) kariesanfälliger Personen wahrscheinlich nicht auf die Hemmung eines einzelnen Fermentes innerhalb der Abbauwege für die Glukose zu suchen ist, sondern auf einer allgemeinen Störung des Kohlenhydratstoffwechsels beruht.

Es bleibt allerdings die Frage offen, welches der disaccharidspaltenden Fermente im Speichel kariesanfälliger Personen an dieser Störung beteiligt ist. Hierfür kommen bekanntlich neben der α-Glukosidase (Maltase) auch die β-Fruktosidasen (β-Fruktofuranosidase, Saccharase, Invertase) in Frage. Es besteht jedoch Grund zur Annahme, daß im Speichel Kariesanfälliger besonders die Maltaseaktivität (α-Glukosidaseaktivität) verringert ist. Die verhältnismäßig langsamere Spaltung der Maltose im Gegensatz zur Saccharose zeigt bei dieser Personengruppe, daß die speziell für die Saccharosespaltung bedeutungsvollen Fermente (β-Fruktofuranosidasen) in der Lage sind, den Unterschied zwischen den Speicheln der beiden Probandengruppen zwar nicht auszugleichen, jedoch zu verringern. (Verhältnis zwischen KA und KR bei Saccharose 1:3, bei Maltose 1:5).

Wenn HARTLES und WASDELL (1954) in ihren Experimenten keine Unterschiede zwischen kariesanfälligen und -resistenten Personen finden konnten, so wird dieses hinreichend dadurch erklärt, daß sie keinen Nüchtern-Ruhespeichel, sondern stimulierten (Paraffin) Nüchternspeichel benutzten. Nach NAUJOKS *et al.* (1961) sind sowohl die Aktivitäten einzelner Fermente als auch die Sauerstoffaufnahme und der Proteingehalt im stimulierten Speichel erheblich geringer als im Ruhespeichel. Nach diesen Autoren nehmen die Fermentaktivitäten und der Proteingehalt auch

Vol. 6
1961

erheblich ab, wenn von derselben Person mehrere Speichelproben unmittelbar hintereinander gemacht werden. Die Unterschiede zwischen kariesanfälligen und -resistenten Personen werden verwischt und verlieren ihre statistische Signifikanz. HARTLESS und WASDELL (1954) benutzten zur Einordnung ihrer Probanden in die beiden Gruppen den DMF-Quotienten. Eine Klassifizierung von Untersuchungspersonen auf Grund des DMF-Index muß jedoch zu Irrtümern führen, da die meisten Menschen im Laufe ihres Lebens bekanntlich keine gleichbleibende Kariesanfälligkeit oder Kariesresistenz zeigen. In der Regel werden vielmehr Zeiträume stärkerer Kariesanfälligkeit von solchen einer mehr oder weniger ausgeprägten Kariesresistenz abgelöst. Ein DMF-Index kann daher eine jederzeit verläßliche Aussage über die Anfälligkeit oder Resistenz einer Person gegenüber der Zahnkaries nicht machen. Zusammenfassend ist festzustellen, daß neben den früher beobachteten, erniedrigten Stoffwechselleistungen im Speichel kariesanfälliger Personen (Sauerstoffaufnahme, Aldolaseaktivität, Hexokinaseaktivität) auch die Spaltung von Saccharose und Maltose verlangsamt ist. Für die Amylaseaktivität sind in dieser Hinsicht bisher nur widersprüchliche Untersuchungsresultate bekannt geworden. Es wäre denkbar, daß eine Störung des Abbaus der Kohlenhydrate im Speichel kariesanfälliger Personen auf der Stufe der Disaccharidspaltung beginnt. Eine verringerte Aktivität der Fermente, welche die nachfolgenden Umsetzungen katalysieren, wäre dann durch ein vermindertes Substratangebot zu erklären. Anderseits besteht die Möglichkeit, daß ein am Ende des Abbauweges der Kohlenhydrate befindlicher Block zu einem Rückstau führt und damit eine verringerte Aktivität der zu Beginn des Abbauweges wirksamen Fermente verursacht. Eine Beantwortung dieser Fragen muß weiteren Untersuchungen vorbehalten bleiben.

LITERATUR

BRAMSTEDT, F., KRÖNCKE, A. und NAUJOKS, R. (1956). Über die Aktivierung des Kohlenhydratabbaus im Speichel durch niedrige Fluorkonzentrationen. *Naturwissenschaften* **43**, 109–110.

BRAMSTEDT, F., KRÖNCKE, A. und NAUJOKS, R. 1957. Über den Einfluss niedriger Fluorkonzentrationen auf den Kohlenhydratabbau im Speichel kariesanfälliger und -resistenter Personen. *Prophylakt. ed.* **1**, 36–39.

BRAMSTEDT, F., KRÖNCKE, A., NAUJOKS, R. und VONDERLINN, R. 1954. Die Verbrennung organischer Substrate im Speichel kariesresistenter und kariesanfälliger Personen. *Dtsch. zahnärztl. Z.* **9**, 782–791.

BRAMSTEDT, R., NAUJOKS, R., und BENEDIKT, I. 1961. "Oral sugar clearance" und fermentaktivitäten im menschlichen Speichel. *Arch. oral Biol.* (Im Druck.)

ERICKSON, Y. 1951. On the salivary Amylase and its significance in the Caries Process. *Acta odont. Scand.* **9**, 89–110.

FISHER, R. A. 1956. In HUDGMAN, C. D., WEAST, R. C. und SELBY, S. M., *Handbook for Chemistry and Physics* (38. Ed.) S. 215–217. Chemical Rubber Publishing Co., Cleveland, Ohio.

FLORENTANO, H. J., FABER, J. E. und JAMES, L. 1941. Studies on the relationship between diastatic activity of saliva and incidence of dental caries. *J. Amer. dent. Ass.* **28**, 1799.

GUSTAFSON, B. E. 1952. Tandkaries ok Kolhydrater. (Teamwork of the Medicinalstyrelsens odontologiska försökstation Vipeholms sjukhus Lund). *Svensk tandlak. Tidskr.* **45**, Suppl.

HARTLES, R. L. und MCDONALD, N. D. 1950. The metabolism of the oral flora—I. The oxygen

uptake and acid production by mixed human saliva in the presence and absence of glucosa. *Biochem. J.* **47**, 60–64.

HARTLES, R. L., SLACK, G. L. und WASDELL, M. R. 1954. The metabolism of the oral flora—III. The utilisation of different carbohydrates by a strain of Lactobacillus isolated from mouth. *Brit. dent. J.* **97**, 67–69.

HARTLES, R. L. und WASDELL, M. R. 1954a. The Fructofuranosidase (Invertase) Activity of Whole Saliva. *J. dent. Res.* **33**, 729 (Abstract).

HARTLES, R. L. und WASDELL, M. R. 1954b. The metabolism of the oral flora—IV. The invertase activity of mixed human saliva. *Brit. dent. J.* **97**, 231–235.

HARTLES R. L. und WASDELL, M. R. 1954c. The metabolism of the oral flora—II. The oxidation of some sugars by mixed human saliva. *Biochem. J.* **55**, 353–355.

HESS, W. C. und SMITH, B. T. 1948. The salivary amylase activity of carious and noncarious individuals. *J. dent. Res.* **27**, 593–598.

KRÖNCKE, A. 1959. *Freie Zucker im menschlichen Nüchternspeichel.* Sammlung MEUSSER, Heft 40, Verlag: Joh. Ambr. Barth, Leipzig.

KRÖNCKE, A. und NAUJOKS, R. 1954. Weitere Ergebnisse zur Entwicklung eines biochemischen Kariestestes. *Dtsch. zahnärztl. Z.* 9, 565–567.

LUNDQVIST, C. 1952. Oral Sugar clearance. *Odont. Revy* **3**, Suppl. 1.

NAUJOKS, R., BRAMSTEDT, F., RANKE, E. und A. KRÖNCKE 1960. Beziehungen zwischen Aldolaseaktivität im Speichel und Karies. *Naturwissenschaften* **47**, 187–188.

NAUJOKS, R., BRAMSTEDT, F., RANKE, E. und KRÖNCKE, A. 1961. Beziehungen zwischen Aldolaseaktivität im Speichel und Karies. (Kongressband VII. Orca-Kongress.) *Arch. oral Biol.* **4**, 171–186.

NAUJOKS, R. und VONDERLINN, R. 1953. Klinische Überprüfung der Möglichkeiten zur Entwicklung eines biochemischen Kariestestes au fder Grundlage des Sauerstoffverbrauches des menschlichen Speichels. *Zahnärztl. Rdsch.* 62, 438–441.

SAUERWEIN, E. 1952. Das Spaltungsvermögen einiger Karieskeime und des Bact. Acidophilum gegenüber verschiedenen Kohlehydraten und deren Reaktion auf das Speichel-pH. *Dtsch. zahnärztl. Z.* **7**, 73–87.

SULLIVAN, J. H. und STORVICK, C. A. 1950. Correlation of saliva analyses with dental examinations of 574 freshmen at Oregon state colleges. *J. dent. Res.* **29**, 165–172.

SWENANDER-LANKE, L. 1957. Influence on salivary sugar of certain properties of foodstuffs and individual oral conditions. *Acta odont. scand.* **15**, Suppl. 23.

TURNER, N. C. und CRANE, E. M. 1944. A relationship between dental caries and saliva. *J. Dent. Res.* **23**, 413–416.

VOLKER, J. F. 1950. Some observations on the action of saliva on sucrose. *J. Dent. Res.* **29**, 680–681. (Abstract).

Vol. 6
1961

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 228–233, 1961. Pergamon Press Ltd.

ÜBER DEN EINFLUSS VON METALLEN AUF DIE GLYKOLYSE IM MENSCHLICHEN SPEICHEL

B. RANKE*

Aus dem biochemischen Labor der Klinik und Poliklinik für Zahn-, Mund- und Kieferkrankheiten der Universität Hamburg, Deutschland

Zusammenfassung—Plättchen von Gold-Edelmetallegierungen und Silberlegierungen mit etwa 200 mm² Gesamtoberfläche sowie Kupfer- und Silberamalgamplättchen mit einer Oberfläche von etwa 100 mm² wurden mit Glukose und menschlichem Speichel inkubiert und der Einfluß der Metalle auf die Glykolyse im Sauerstoffaufnahmetest beobachtet.

Während bestimmte Gold-Edelmetallegierungen mit einem Kupfergehalt von 1, 5, 4 und 9 Prozent die Sauerstoffaufnahme kaum beeinflußten, bewirkten eine Silber–Palladiumlegierung eine Hemmung von etwa 10 Prozent und eine Silber–Cadmium–Kupferlegierung eine solche von etwa 50 Prozent. Kupferamalgam hatte eine durchschnittliche Hemmungsaktivität von 86 Prozent. Die Silberamalgamplättchen zeigten in Abhängigkeit von der Zeitspanne seit der Amalgambereitung und nach Charge einen mehr oder weniger starken Hemmeffekt. Innerhalb der ersten 3 Wochen lagen die Extremwerte zwischen 0 und 77 Prozent. Die Bedeutung der Glykolysehemmung wird diskutiert.

Abstract—Small plates of gold–rare–metal alloys and of silver alloys with a surface area of about 200 mm² as well as plates of copper- and silver-amalgam with a surface area of about 100 mm² have been incubated with glucose and human saliva, and the influence of the metals on glycolysis has been observed by the oxygen uptake test.

While certain gold–rare–metal–alloys containing 1, 5, 4 and 9 per cent copper scarcely changed the oxygen uptake, a silver–palladium alloy exhibited an inhibition activity of about 10 per cent as did a silver–cadmium–copper alloy of about 50 per cent in average. The copper amalgam had an inhibition activity of 86 per cent in average. The plates of silver amalgam showed an inhibition dependent of batch and time elapsed since preparation. In the saliva specimens investigated the inhibition of oxygen uptake was found to range from 0 to 77 per cent during the first three weeks of amalgam age. The significance of an inhibition of the glycolysis is discussed.

DA seit langem bekannt ist, daß Metalle Fermente hemmen oder aktivieren können (WARBURG, 1948), lag die Frage nahe, ob und in welchem Ausmaß das Mundhöhlenmilieu durch die in der konservierenden Zahnheilkunde gebräuchlichen Metalle beeinflußt wird. Die großen individuellen Schwankungen des Speichels im Verhalten gegenüber biochemischen Testen, die das Studium des Speichels erschweren, könnten zum Teil durch das Vorhandensein metallischen Zahnersatzes

*Wir danken der Deutschen Forschungsgemeinschaft für finanzielle Unterstützung.

und von Metallfüllungen bedingt sein. Nach Zahnbehandlungen wurde bemerkenswerterweise eine erhebliche Änderung des Mundhöhlenmilieus bezüglich des Vorkommens von Lactobacillen festgestellt (LAMMERS und HAFER, 1956). SHEPPARD (1935) prüfte aus gezogenen Zähnen gebrochene Metallfüllungen und reine Metalle auf ihre Bakterizidie gegenüber einigen Speichelbakterien und stellte fest, daß reines Gold und Silber keine bakteriziden Eigenschaften aufwiesen, sondern oftmals eine Aktivierung des Bakterienwachstums verursachten. Reines Kupfer und Quecksilber dagegen waren stark bakterizid. Amalgamfüllungen variierten erheblich in ihren bakteriziden Eigenschaften. Neuere Arbeiten über den Einfluß von Metallen auf das Wachstum von Mundbakterien sind uns nicht bekannt geworden.

Vol. 6
1961

MATERIAL UND METHODEN

Unser Interesse galt der Beeinflussung der Speichelglykolyse durch Metalle. Als Testmethode erschien uns der Sauerstoffaufnahmetest im WARBURG- Apparat (UMBREIT, BURRIES und STAUFFER, 1957) am besten geeignet.

Das Gesamtvolumen des Ansatzes war 2,5 ml und setzte sich zusammen aus:

1,25 ml Versonal-Na-puffer pH 6,9 ($5{,}36 \times 10^{22}$M)
0,50 ml Speichel
0,25 ml Glukose (0,1 M in bidest. Wasser)
bidest. Wasser ad 2,5 ml

Zur Bindung des freiwerdenden CO_2 enthielt das Zentralgefäß 0,3 ml 10% NaOH.

Bei den Testproben wurden ein oder zwei handelsübliche Gußwürfel* mit einer Gesamtoberfläche von je etwa 100 mm² für die Dauer der Inkubation zugesetzt. Die Versuchszeit betrug 60 Minuten bei 37°C und einer Schüttelfrequenz von etwa 120/min.

Die Kupfer- und Silberamalgame wurden nach den in Deutschland üblichen Verfahren hergestellt und in Plastikformen gestopft, die größenmäßig genau den Gußwürfeln entsprachen. Das Kupferamalgam wurde in kleinen Plätchen vom Fachhandel bezogen, die nach Erhitzung verrieben und dann gestopft wurden. Für die Zubereitung des Silberamalgams diente Zinn-Silberfeilung und Quecksilber. Mittels eines Dosators abgemessene Mengen wurden 20 Sekunden in einem Schüttelgerät angemischt und dann gestopft. Die so hergestellten Amalgamkörper wurden nach 24 Stunden aus den Formen gebrochen, nach 48 Stunden poliert sowie kurz vor jedem Versuch mit Bürste und Kreide gereinigt und mit bidestilliertem Wasser gespült. In den Zeiten zwischen den Versuchen wurden sie unter Wasser aufbewahrt.

Als Untersuchungsmedium diente unstimulierter Nüchternspeichel nach RANKE und RANKE (1961), produziert unmittelbar nach dem morgendlichen Erwachen.

*Für die Zurverfügungsstellung der Metallegierungen danken wir der Deutschen Gold- und Silber-Scheideanstalt, Hamburg.

Da wir Versuchsreihen mit Speichel jeweils derselben Person über längere Zeit planten und dafür eine möglichst gleiche Speichelzusammensetzung erwünscht war, wurden der Zuverlässigkeit halber vorwiegend Speichelproben des Autors und der Laboratoriumsmitglieder für den Test herangezogen.

ERGEBNISSE

Die Resultate der vergleichenden Untersuchungen über die Wirkungen bestimmter Gold–Edelmetallegierungen, Silberlegierungen sowie von Kupfer- und Silberamalgamen auf die Speichelglykolyse sind in Tabelle 1 zusammengestellt. Bei den

TABELLE 1. HEMMUNG DER SAUERSTOFFAUFNAHME DURCH VERSCHIEDENE METALLEGIERUNGEN UND AMALGAME IM MENSCHLICHEN NÜCHTERNSPEICHEL

Metallprobe	Alter der Metallprobe	Hemmung (%)	Metalloberfläche (mm^2)
Goldlegierung mit 1,5% Kupfer	∞	± 5	200
Goldlegierung mit 4% Kupfer	∞	± 5	200
Goldlegierung mit 9% Kupfer	∞	± 5	200
Silber–Palladium-Legierung	∞	9–13	200
Silber–Cadmium–Kupfer–Legierung	∞	25–57	200
Kupfer–Amalgam	0–8 Wochen	74–91	100
Silber–Amalgam	0–3 Wochen	0–77	100

Edelmetallegierungen ist das Alter unbekannt und mit ∞ gekennzeichnet. Die Verwendung von zwei Plättchen im Test mit einer Oberfläche von zusammen etwa 200 mm^2 dürfte etwa einer großen oder zwei mittleren Füllungen entsprechen. Bei den Amalgamen hielten wir dagegen die Verwendung von nur einem Plättchen mit etwa 100 mm^2 Oberfläche für sinnvoll. Drei untersuchte Gold-Edelmetallegierungen, die u.a. im Kupfergehalt unterschiedlich sind, zeigten von Speichel zu Speichel einen unterschiedlichen (teils aktivierenden, teils hemmenden) Effekt von nicht mehr als 5 Prozent. Eine Silber-Palladium-Legierung offenbarte eindeutig eine hemmende Wirkung von etwa 10 Prozent (9–13 Prozent), während eine Silber–Cadmium-Legierung, die heute allerdings kaum mehr verwendet wird, im Durchschnitt eine hemmende Wirkung von 50 Prozent hatte. Von den Amalgamen zeichnete sich das Kupferamalgam durch besonders starke Hemmung der Glykolyse aus. Die durchschnittliche Wirkung betrug 86 Prozent mit Extremwerten von 74 und 91 Prozent. Durch den Fachhandel vertriebene Kupferamalgamrohwürfel unbekannten Alters von etwa gleichgroßer Oberfläche hatten die gleiche Hemmungsaktivität. Die hemmende Wirkung des Silberamalgams erwies sich gegenüber der des Kupferamalgams als sehr unterschiedlich. Innerhalb von 3 Wochen nach der Bereitung des Amalgams wurden Hemmungsaktivitäten von 0 bis 77 Prozent beobachtet, wobei die Aktivität von Charge zu Charge variierte.

Um zu ermitteln, ob sich die Aktivität des Silberamalgams mit dem Alter des Amalgams ändert, wurden 6 Versuchsreihen durchgeführt, bei denen über eine Zeitspanne von 3 Wochen im Abstand von 1 bis 4 Tagen und nach einigen Wochen die Aktivität derselben Amalgamplättchen im Speichel derselben Person geprüft wurde. In Abb. 1 sind die Hemmungsaktivitäten der Amalgamplättchen im Speichel

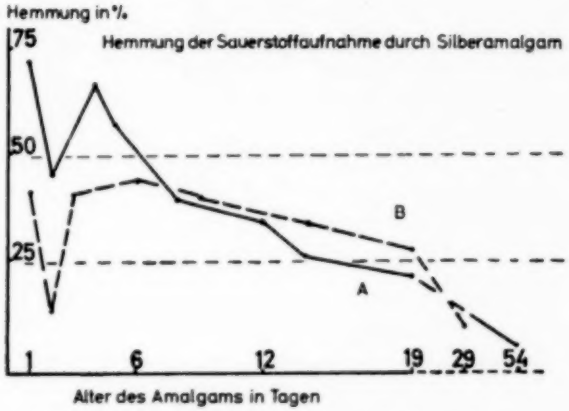


ABB. 1. Hemmung der Sauerstoffaufnahme durch Silberamalgam im Nüchternspeichel zweier Personen A und B in Abhängigkeit vom Alter des Amalgams. Jeder Punkt bedeutet den Mittelwert der Aktivität von zwei voneinander unabhängigen, zeitlich hintereinander liegenden Meßreihen mit verschiedenen Amalgamchargen.

des Autors (Kurve A) und einer Assistentin (Kurve B) in Abhängigkeit vom Alter des Amalgams dargestellt. Jeder Punkt bedeutet den Mittelwert der Aktivität von zwei voneinander unabhängigen, zeitlich hintereinander liegenden Meßreihen mit verschiedenen Amalgamchargen. Eine Tendenz des Abfalls der Aktivitäten mit der Zeit ist deutlich zu erkennen. Die auffällige Verminderung der Aktivität am zweiten Tage hängt wahrscheinlich mit dem unmittelbar vorausgehenden Polieren der Plättchen zusammen. Sie war auch im Speichel anderer Versuchspersonen mit anderen Amalgamchargen nachzuweisen. Nach dem Polieren nahm die Aktivität durchweg für kurze Zeit zu, um dann mehr oder weniger schnell abzufallen, aber nicht völlig zu verschwinden. In dieser Versuchsreihe wurden noch nach zwei Monaten Hemmungsaktivitäten von 5–10 Prozent nachgewiesen. In den Versuchsreihen mit anderen Personen waren die Schwankungen der Hemmungsaktivitäten von einer Probe zur nächstfolgenden sehr viel größer, eine Beobachtung, die auf die Schwierigkeiten hinweist, die Bedingungen, unter denen die Proben entnommen werden, konstant zu halten. Sie deutet ferner darauf hin, daß die Bakterienflora sich möglicherweise innerhalb kurzer Zeit ändert. Die Durchschnittswerte dieser Versuchsreihen sind

für den 1. bis 3. Tag 30,1 ± 6,1 Prozent,
für den 5. bis 8. Tag 36,7 ± 6,7 Prozent,
für den 10. bis 14. Tag 25,5 ± 6,0 Prozent und
für den 19. bis 24. Tag 12,1 ± 6,6 Prozent,

wobei die Abweichungen den mittleren Fehler des Mittelwertes darstellen.

Die eben beschriebenen Versuchsreihen sprechen dafür, daß die Hemmungsaktivität mit dem Alter des Amalgams abnimmt, wenn dieselben Amalgamplättchen innerhalb einer Versuchsreihe getestet werden. Weitere Versuche ergaben, daß mit Silberamalgamplättchen aus verschiedenen Chargen eine Beziehung zwischen Hemmungsaktivität und Amalgamalter nicht zu ermitteln war.

DISKUSSION

Es wurden "erste Nüchternspeichel" als Probenmaterial verwandt (RANKE und RANKE, 1961; BRAMSTEDT, KRÖNCKE und NAUJOKS, 1957), weil nur diese die Gewähr für möglichst konstante Bedingungen bieten und ein Mundhölenmilieu reflektieren, das sich während der Nachtruhe einstellt. Die Zeit der Nachtruhe ist aber für die Karies von großer Bedeutung, da nach PRADER (1956) sich der Hauptangriff der gebildeten Säuren am Zahnschmelz besonders während der Nacht ungestört auswirken kann. Während die vergleichenden Untersuchungen von handelsüblichen Gold-Edelmetallegierungen keinen nennenswerten Einfluß im Speichel unserer Probanden erkennen ließen, offenbarten neben den heute weniger gebräuchlichen Silberlegierungen die Amalgame zum Teil erhebliche Hemmwirkungen. Trotz einiger Abweichungen von den *in vivo* vorliegenden Verhältnissen glauben wir aus den Versuchsergebnissen schließen zu können, daß die Glykolyse auch *in vivo* in stärkstem Maße durch die Gegenwart von Kupferamalgam und mehr oder weniger stark durch Silberamalgamfüllungen gehemmt werden kann. Bei letzteren dürfte die Art der Zubereitung neben dem Alter des Amalgams für die Hemmwirkung von großer Bedeutung sein. Untersuchungen, inwieweit das Mischungsverhältnis von Feilung und Quecksilber und der Stopfdruck für die Hemmungsaktivität bestimmend sind, sind im Gange. Selbstverständlich stellt die Größe der Metalloberfläche einen entscheidenden Faktor dar. So wurde z.B., bei einem Versuch mit Silberamalgam die Hemmung durch Hinzufügen eines zweiten Plättchens von 65 auf 90 Prozent gesteigert. Unsere Versuchsergebnisse stehen bezüglich der Kupfer-und Silberamalgamwirkung im Einklang mit den von SHEPPARD (1935) an Bakterienkulturen gemachten Erfahrungen.

Wir glauben annehmen zu können, daß nach umfangreichen Gebißsanierungen mit Amalgamen (spez. Kupferamalgamen) die Glykolyse im Speichel während der Nachtstunden erheblich gehemmt werden kann. Folge der Hemmung wäre eine Verzögerung der "oral sugar clearence" (LUNDQVIST, 1952), d.h. die Verweildauer der Kohlenhydrate in der Mundhöhle würde verlängert. Auf Grund der Untersuchungsergebnisse von LUNDQVIST (1952), PRADER (1956), SWENANDER-

Vol. 196

LANKE (1957), BRAMSTEDT *et al.* (1954, 1957) und NAUJOKS *et al.* (1961), würde die Verzögerung der "oral sugar clearance" die Tendenz zur Kariesanfälligkeit verstärken.

LITERATUR

BRAMSTEDT, F., KRÖNCKE, A. und NAUJOKS, R. 1957: Über den Einfluß von Fluor auf den Glukoseabbau isolierter Mundbakterienstämme. ORCA Yearbook 1957, 201–206.

BRAMSTEDT, F., KRÖNCKE, A., NAUJOKS, R. und VONDERLINN, R. 1954. Biochemische Speicheluntersuchungen—VI. Die Verbrennung organischer Substrate im Speichel kariesresistenter und kariesanfälliger Personen. *Dtsch. zahnärztl. Z.* **9**, 782–791.

ERICSSON, Y. 1961. Die Beziehungen zwischen Speichel und Karies. *Dtsch. zahnärztl. Z.* **16**, 164–170.

LAMMERS, T. und HAFER, H. 1956. *Biologie der Zahnkaries*. Dr. Alfred Hüthig Verlag, Heidelberg.

LUNDQVIST, C. 1952. Oral sugar clearance. Its influence on dental caries activity. *Odont. Revy* **3**, Suppl. 1,

NAUJOKS, R., BRAMSTEDT, F., RANKE, E. und AUERBACH, M. 1961. Zur Problematik der Speichelgewinnung für biochemische Untersuchungen. *Dtsch. zahnärztl. Z.* **16**, 171–175.

PRADER, F. 1956. Der Brotabbau durch Speichel. *Schweiz. Mschr. Zahnheilk.* **66**, 210–221.

RANKE, E. und RANKE, B. 1961. The protein and sediment contents of whole unstimulated human saliva in caries resistant and caries susceptible individuals. Vortrag **9**. ORCA—Jahrestagung London, 1961.

SHEPPARD, M. S. 1935. The bactericidal action of pure metals and metal fillings. *Dent. Cosmos* **77**, 968–975.

SWENANDER-LANKE, L. 1957. Influence on salivary sugar of certain properties of food stuffs and individual oral conditions. *Acta odont. scand.* **15**, Suppl. 23.

UMBREIT, W. W., BURRIES, R. H. und STAUFFER, J. F. 1957. *Manometric Techniques*. Burgess, Minneapolis.

WARBURG, O. 1948. *Schwermetalle als Wirkungsgruppen von Fermenten*. Verlag Dr. Werner Saenger, Berlin.

Vol. 6
1961

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 234-240, 1961. Pergamon Press Ltd.

ÜBER DEN SEDIMENT- UND PROTEINGEHALT DES SPEICHELS KARIESANFÄLLIGER UND KARIESRESISTENTER PERSONEN

E. RANKE und B. RANKE*

Aus dem biochemischen Labor der Klinik und Poliklinik für Zahn-, Mund- und Kieferkrankheiten der Universität Hamburg, Deutschland

Zusammenfassung—Es wurden Protein- und Sedimentbestimmungen im unstimulierten Nüchternspeichel durchgeführt. Der Proteingehalt wurde nach der Biuretmethode, der Sedimentgehalt durch 30 Minuten Zentrifugieren in geeichten Hämatokritröhrchen nach WINTROBE bei 4500 U/min bestimmt.

Die Ergebnisse ließen mit hoher Signifikanz einen geringeren Proteingehalt und geringeren Sedimentgehalt des Speichels kariesanfälliger Personen gegenüber resistenten erkennen, wenn die ersten zwei unstimulierten, morgendlichen Nüchternspeichelproben von je 4 ml verglichen wurden.

Untersuchungen über Proteingehaltsschwankungen im Speichel im Verlauf eines Tages zeigten sowohl bei Personen, die während der Zeitdauer des Versuchs nüchtern blieben, als auch bei Personen, die Mahlzeiten einnahmen, ein steiles Absinken der ersten hohen Nüchternwerte von etwa 15–18 mg auf ein relativ konstantes Niveau von 1–5 mg Protein/ml Speichel bis zum Abbruch der Versuche am Nachmittag.

Abstract—Protein and sediment determinations have been carried out in unstimulated fasting early morning saliva specimens. The protein contents have been determined according to the Biuret method; the sediment contents have been measured after centrifuging in gauged hematocrit tubes according to Wintrobe for 30 min at 4500 rev/min. The results revealed, with high significance, lower protein levels as well as smaller sediment contents with the caries susceptible group against the resistant group, if the first two unstimulated fasting early morning saliva specimens of 4 ml each were compared.

Investigations concerning the variations of the protein contents during the course of a day showed with fasting individuals, and with individuals with normal food intake likewise, a steep decline from the first high value of the fasting specimens of about 15–18 mg/ml to much lower ones of a relatively constant remaining level of about 1–5 mg of protein/ml of saliva up to the interruption of the essay in the afternoon.

UNTERSUCHUNGEN über eine eventuelle Beziehung zwischen Proteingehalt des Speichels und Zahnkaries wurden u.a. von KRASNOW und OBLATT (1933) durchgeführt. Die Autoren fanden, daß der Proteingehalt des gesamten Speichels bei kariesresistenten Personen häufig erhöht war, daß aber nach Zentrifugieren des Speichels der gleichen Gruppe weniger Sediment abgesetzt wurde als bei kariesanfälligen Personen. Sie vermuteten, daß das Protein im Speichel kariesresistenter Personen

*Wir danken der Deutschen Forschungsgemeinschaft für finanzielle Unterstützung.

stabiler sei als bei kariesanfälligen. GELLER und ROVELSTAD (1959) beobachteten einen leicht erhöhten Gehalt an Gesamtprotein im Parotisspeichel von Kariesanfälligen. DEWAR und PARFITT (1954) stellten entgegen der landläufigen Ansicht, daß hohe Speichelviskosität mit erhöhter Kariesanfälligkeit verbunden ist, keine Relation zwischen Mucingehalt und Karies fest. Die sich widersprechenden Angaben über den Proteingehalt des Speichels in Beziehung zur Karies beruhen sicher zum Teil auf der Anwendung unterschiedlicher Methoden der Proteinbestimmung und der Speichelgewinnung. Die Ergebnisse der Speichelproteinbestimmungen im Rahmen unserer biochemischen Speicheluntersuchungen an kariesresistenten und kariesanfälligen Personen sollen zu rKlärung der Begriffe beitragen und die Kenntnisse über die Veränderungen des Mundhöhlenmilieus erweitern.

Vol. 6
1961

MATERIAL, METHODEN

Speichelproben kariesanfälliger und -resistenter Personen wurden morgens unmittelbar nach dem Erwachen, unstimuliert, nüchtern und vor jeglicher Mundreinigung gewonnen. Am Abend vor der Speichelgewinnung wurde von den Versuchspersonen der Mund nur mit Wasser ausgespült, also weder Zahnpflegemittel noch Bürste benutzt. Die gesammelte Speichelmenge betrug durchschnittlich 4 ml. Der Speichel war den Versuchsbedingungen nach ein sogenannter Mischspeichel und kein reiner Drüsenspeichel. Die Auswahl der Versuchspersonen (vorwiegend Angehörige der Universitätsklinik und Poliklinik für Zahn-, Mund- und Kieferkrankheiten Hamburg) erfolgte auf Grund des klinischen Zustandes des Gebisses, der Kariesanamnese und wurde durch einen O_2-Aufnahmetest des Speichels unterstützt (KRÖNCKE und NAUJOKS, 1956).

Frühere Untersuchungen hatten gezeigt, daß eine Sauerstoffaufnahme über 95 μl O_2/ml Speichel Kariesresistenz, eine solche unter 65 μl O_2 Kariesanfälligkeit anzeigen (KRÖNCKE und NAUJOKS, 1956). Zur Sedimentbestimmung wurden geeichte Hämatokritröhrchen nach WINTROBE benutzt. Nach 30 Minuten Zentrifugieren bei 4500 U/min wurde die Sedimentmenge abgelesen und auf 1 ml Speichel berechnet. Anschließend wurde der Inhalt dieser Röhrchen vorsichtig in Versuchsröhrchen übergeführt und der Proteingehalt vermittels der Biuretmethode (ROBINSON und HOGDEN, 1940) bestimmt. Um die zum Teil starke Trübung der Speichellösung, die die Extinktion erheblich erhöht, zu eliminieren, wurde zu jeder Bestimmung außer der Reagenzblindprobe eine Speichelprobe mit 0,2 N NaOH statt des Biuretreagenzes angesetzt und diese Extinktion von derjenigen der eigentlichen Versuchs. lösung abgezogen. Die Extinktionswerte wurden anhand einer Eichkurve auf mg Casein berechnet und auf 1 ml Speichel bezogen.

ERGEBNISSE

Um ein Maß für die Schwankungen in der Speichelzusammensetzung während eines Tages bei ein und derselben Person zu erhalten, wurden in einem Vorversuch bei kariesresistenten und -anfälligen Personen Speichelproben im Abstand von

½ bis 1½ Stunden gesammelt. Wie das in Tabelle 1 für eine kariesresistente Person angeführte repräsentative Beispiel erkennen läßt, fand sich der höchste Proteinwert im ersten Nüchternspeichel (S1). Steiles Absinken des Proteingehaltes auf weniger als die Hälfte des Anfangswertes zeigte die zweite Speichelprobe (S2).

TABELLE 1. SPEICHELPROTEINWERTE EINER KARIESRESISTENTEN PERSON IM VERLAUF EINES VORMITTAGS (NÜCHTERN) UND EINES TAGES MIT NORMAL EINGENOMMENEN MAHLZEITEN

Probe	Uhrzeit	mg Protein/ ml Speichel	Probe	Uhrzeit	mg Protein/ ml Speichel
S1	6^{50}	18,39	S1	7^{00}	13,04
S2	7^{20}	7,98	T1*†	8^{45}	2,48
S3	7^{50}	2,64	T2	9^{20}	3,04
S4	8^{20}	5.13	T3	10^{20}	3,32
S5	9^{10}	2,91	T4	11^{20}	4,32
S6	9^{50}	3,25	T5*	12^{20}	2,20
S7	10^{20}	2,20	T6	13^{20}	3,32
S8	11^{20}	3,11	T7*	15^{15}	1,94
S9	12^{20}	5,04	T8*†	16^{15}	2,76
S10	13^{00}	2,67			
T1*	14^{00}	1,88			

* = 30 Minuten nach dem Essen.
*† = 90 Minuten nach dem Essen.

Von der dritten Speichelprobe ab wurde für beide Versuchsgruppen ein Kurvenverlauf gefunden, bei dem die Proteinwerte bis zum Versuchsende am Nachmittag zwischen 1,8 und 5,2 mg Protein/ml Speichel lagen. Nur die ersten Speichelproben der beiden Personengruppen erwiesen sich als signifikant unterschiedlich. Nach einer Mahlzeit gewonnene Speichelproben schienen etwas niedrigere Proteinwerte zu ergeben, wenn sie 30 Minuten nach der Nahrungsaufnahme mit anschließender Mundspülung gesammelt wurden.

Da der Vorversuch erkennen ließ, daß vom dritten Nüchternspeichel ab Unterschiede im Proteingehalt des Speichels kariesresistenter und -anfälliger Personen innerhalb der individuellen Streuung lagen, wurden in einem zweiten Versuch vergleichende Proteinanalysen in drei unmittelbar hintereinander produzierten Nüchternspeichelproben von je 4 ml bei 20 Kariesresistenten, 18 Kariesanfälligen und 14 nicht sicher zu klassifizierenden Personen durchgeführt und durch Sauerstoffaufnahmeteste ergänzt. Die erste Nüchternspeichelprobe (S1), deren Gewinnung oben beschrieben ist, zeigte die größten Unterschiede im Proteingehalt (vergl. Tabelle 2). Der unmittelbar im Anschluß an die erste Nüchternspeichelprobe (S1) gesammelte Speichel (S2) ergab ebenfalls noch signifikante Unterschiede. Der Nüchternspeichel kariesanfälliger Personen erwies sich unter diesen Bedingungen

einwandfrei als proteinärmer als der kariesresistenter. Die Speichelproteinwerte der nicht zu klassifizierenden Personen lagen zwischen den beiden extremen Gruppen. Die Speichelproben S3 zeigten keine statistisch gesicherten Unterschiede.

TABELLE 2. PROTEINGEHALT und O_2-VERBRAUCH PRO ml SPEICHEL IN DEN ERSTEN DREI UNMITTELBAR AUFEINANDER FOLGENDEN NÜCHTERNSPEICHELPROBEN VON JE 4 ml BEI KARIESRESISTENTEN, -ANFÄLLIGEN UND -FRAGLICHEN PERSONEN. DIE Ergebnisse sind Durchschnittswerte für Versuchsreihen von 20 (KR), 18 (KA) bzw. 14 (K†) Einzelversuchen

mg Protein/ml Speichel

Probe	KR	KA	K†	*t*-Wert (KR : KA)
S1	19,7 ± 1,58*	7,6 ± 0,76*	12,2 ± 1,35*	6,88
S2	7,8 ± 1,15	3,0 ± 0,42	6,1 ± 0,97	3,20
S3	4,5 ± 1,02	2,3 ± 0,50	3,1 ± 0,42	1,92

Sauerstoffverbrauch (μl O_2/ml Speichel pro Stunde)

Probe	KR	KA	K†	*t*-Wert (KR : KA)
S1	156,3 ± 11,8*	43,4 ± 2,9*	74,2 ± 2,0*	9,29
S2	68,3 ± 6,6	25,7 ± 2,4	45,4 ± 4,8	6,07
S3	50,7 ± 5,3	23,2 ± 3,6	40,3 ± 4,6	4,31

* = Mittlerer Fehler des Mittelwertes.

Vergleichende Protein- und Sedimentbestimmungen im ersten Nüchternspeichel von 22 kariesresistenten, 19 kariesanfälligen und 10 kariesfraglichen Personen bestätigten den bei Kariesanfälligkeit beobachteten geringeren Proteingehalt und zeigten, daß auch der Sedimentgehalt bei kariesanfälligen Personen signifikant erniedrigt ist (vergl. Tabelle 3). Die Werte der Kariesfraglichen liegen zwischen den beiden extremen Gruppen.

TABELLE 3. SEDIMENT- UND PROTEINGEHALTE DER ERSTEN NÜCHTERNSPEICHELPROBE (4 ml) VON KARIESRESISTENTEN, -ANFÄLLIGEN UND -FRAGLICHEN PERSONEN. Die Ergebnisse sind Durchschnittswerte für Versuchsreihen von 22 (KR), 19 (KA) und 10 (K†) Einzelversuchen

	KR	KA	K†	*t*-Wert (KR : KA)
ml Sediment/ ml Speichel	0,186 ± 0,014*	0,086 ± 0,010*	0,120 ± 0,013*	5,85
mg Protein/ ml Speichel	16,00 ± 0,88	6,48 ± 0,65	9,60 ± 1,05	10,13

* = Mittlerer Fehler des Mittelwertes.
† = Kariesfraglich

Um festzustellen, wie sich der Proteingehalt auf die beiden Fraktionen des zentrifugierten Speichels verteilt, wurden Sediment und Überstand getrennt untersucht. Bei Kariesanfälligen zeigte sich der Proteingehalt, der auf den Sedimentanteil des Speichels pro ml Speichel entfällt, signifikant niedriger als bei resistenten Personen:

KA = 3,20 mg/ml Speichel

KR = 13,88 mg/ml Speichel

Im Gegensatz dazu sind die Proteingehalte, die auf den Überstand pro ml Speichel entfallen, nicht signifikant unterschiedlich (vergl. Tabelle 4).

TABELLE 4. PROTEINGEHALT IM SEDIMENT UND ÜBERSTAND DES SPEICHELS KARIESANFÄLLIGER UND -RESISTENTER PERSONEN (BIURETMETHODE)

mg Protein/ml Nüchternspeichel

KA			KR		
	Sed.	Überst.		Sed.	Überst.
B.R.	4,85	2,45	Sch.	12,90	3,41
G.G.	4,43	2,02	B.B	9,81	7,25
O.S.H.	0,69	0,88	Frl. E.	10,81	4.94
Frl. K.	5,94	3,17	O.S.	20,40	3,29
Fr. A.	4,63	0,40	T. Sch.	16,90	1,62
I.B.	3,61	3,99	I.M.	6,80	3,22
Fr. G.	0,21	2,59	H.R.	23,00	2,00
E.R.	2,96	2,96	A.M.	8,00	2,46
T.P.	1,50	0,61	M.R.	19,05	1,00
			I.W.	13,04	3,28
D =	3,20 ± 0,668	2,12 ± 0,415		13,88 ± 1,590	3,28 ± 0,512

t-Wert für KA/KR Sediment: 6,32
KA/KR Überstand: 1,74

STATISTIK

Die statistische Sicherung der Unterschiede zwischen den Mittelwerten wurde nach den bei HODGMAN (1956) angegebenen Methoden durchgeführt. Danach ist

$$t = \bar{x}_1 - \bar{x}_2 \left[\frac{i\,(x_i - \bar{x}_1)^2 + i\,(x_{2i} - \bar{x}_2)^2}{N_1 + N_2 - 2} \left(\frac{1}{N_1} + \frac{1}{N_2} \right)^{-1} \right]$$

bei $N_1 + N_2 - 2$ Freiheitsgraden.

DISKUSSION

Die erzielten Ergebnisse zeigen, daß eine standardisierte Probeentnahme für Speicheluntersuchungen von grundlegender Wichtigkeit ist und sich nur die erste unstimulierte Nüchternspeichelprobe in einer Menge von etwa 4 ml für solche Experimente eignet. Alle später gesammelten Proben zeigen zum Teil stark erniedrigte Werte z.B., der Aldolaseaktivität, der Sauerstoffaufnahme und des Proteingehaltes (NAUJOKS *et al.* 1961). Die Unterschiede zwischen den nach dem klinischen Zustandsbild und der Sauerstoffaufnahme als kariesanfällig und kariesresistent klassifizierten Personen sind hier nicht mehr signifikant und können sich beim Vergleich verschiedener Speichelfraktionen sogar umkehren. Nach den drei ersten Nüchternspeichelproben von je 4 ml erreichen die Proteinwerte sowohl bei weiterhin nüchternen Personen als auch nach zu normalen Zeiten eingenommenen Mahlzeiten ein verhältnismäßig konstantes Niveau von etwa 2 bis 5 mg Protein pro ml Speichel. Signifikante Unterschiede zwischen KA und KR sind dann nicht mehr festzustellen. Die gefundenen Werte für diese Fraktionen entsprechen den von

GORLIN (1948) (280 mg/100 ml)
KRASNOW und KARSHAN (nach GORLIN, 1948) (260–300 mg/100 ml)
HARNDT (1959) (0–560 mg/100 ml)

angegebenen Mengen.

Diese Zahlen weisen darauf hin, daß bei den Versuchen der angeführten Autoren nicht die von uns verwendete erste Speichelprobe (S1) analysiert wurde, sondern etwa S4 oder eine spätere. Auch KRASNOW und OBLATT (1933) wiesen schon darauf hin, daß Unterschiede zwischen KA- und KR-Personen am deutlichsten festzustellen sind, wenn unstimulierter Nüchternspeichel für die Versuche verwendet wird. Diese Feststellungen konnten durch die vorliegenden Untersuchungen sowie durch die Experimente von NAUJOKS *et al.* (1961) u.a. im einzelnen bestätigt werden.

Unterschiede in der Probeentnahme wären danach bereits eine Erklärung für die in der Literatur zu findenden, häufig voneinander abweichenden oder sogar sich widersprechenden Ergebnisse. Die Frage, ob der gefundene höhere Proteingehalt im Speichel kariesresistenter Personen im Hinblick auf eine Erhöhung der Pufferkapazität des Speichels von Bedeutung ist, konnte auf Grund der vorliegenden Ergebnisse nicht beantwortet werden. Die sehr einfache Bestimmung des Speichelsedimentes erscheint jedoch geeignet, die Klassifizierung der zu untersuchenden Personengruppen zu erleichtern.

LITERATUR

DEWAR, M. R. und PARFITT, G. J. 1954. Mucin contents, physical property of saliva and caries activity. *J. dent. Res.* **33**, 751–756.

ERICSSON, Y. 1949. Enamel-apatite solubility. *Acta odont. scand.* **8**, Suppl. 3, 1–139.

GELLER, J. H. und ROVELSTAD, G. H. 1959. Electrophoresis of saliva. *J. dent. Res.* **38**, 1060–1065.

GORLIN, R. 1948. Variations in salivary protein concentration. *J. dent. Res.* **27**, 603–608.

HARNDT, R. 1959. Eiweißgehalt des Parotisspeichels. *Dtsch. Zahn-, Mund-, Kieferhk.* **30**, 110–121.

KARSHAN, M. 1938. Factors in unstimulated saliva correlated with presence and activity of dental caries. *J. dent. Res.* **17**, 328–329.

KRASNOW, F. und OBLATT, E. B. 1933. Studies of Variations in salivary Proteins. *J. dent. Res.* **13**, 239 (Abstract).

KRÖNCKE, A. und NAUJOKS, R. 1956. Dental caries susceptibility tests and their significance in dental practice. *Int. dent. J., Lond.* **6**, 174–188.

NAUJOKS, R., BRAMSTEDT, F., RANKE, E. und AUERBACH, M. 1961. Zur Problematik der Speichelgewinnung für biochemische Untersuchungen. *Dtsch. zahnärztl. Z.* **16**, 171–175.

ROBINSON, H. W. und HOGDEN, C. G. 1940. The Biuret Reaction in the determination of serum proteins—II. *J. biol. Chem.* **135**, 727–731.

SAITO, S. und KIZU, K. 1959. Phosphatase activity in whole and parotid saliva and its relationship to dental caries. *J. dent. Res.* **38**, 500–505.

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 241–248, 1961. Pergamon Press Ltd.

BEZIEHUNGEN ZWISCHEN DEM PHOSPHATGEHALT DES SPEICHELS UND KARIES*

G. AHRENS
Biochemisches Laboratorium der Klinik und Poliklinik für Zahn-, Mund- und Kieferkrankheiten, Hamburg, Deutschland

Zusammenfassung—Im Nüchternspeichel von kariesanfälligen und kariesresistenten Personen wurden folgende Phosphatfraktionen untersucht: Gesamtphosphat, anorganisches Phosphat, nicht säurelösliches und säurelösliches organisches Phosphat. Es konnte gezeigt werden, daß

1. eine Korrelation aller Fraktionen mit der Sauerstoffaufnahme des Speichels besteht,
2. alle Fraktionen im Speichel Kariesresistenter signifikant erhöht sind,
3. alle Fraktionen untereinander in einem bestimmten Verhältnis stehen.

Abstract—From the literature which has so far been published it is not possible to draw definite conclusions about the relation between the phosphate content of human saliva and caries; this paper therefore examines these relations from a new point of view.

The examination concerned the amount of oxygen uptake of human saliva as well as the following phosphate fractions—total phosphate (i.e. the sum of all organic and inorganic phosphate compounds), total phosphate in de-albumenized supernatant and inorganic phosphate. From these data the values for organic phosphate which is soluble in acid and for non-acid soluble organic phosphate were calculated. Great care was taken to obtain the saliva of a person under certain carefully described conditions.

The results show a definite correlation between the oxygen uptake and all kinds of phosphates. Dividing the test persons into caries active persons and caries resistant ones, we obtain the following average values whose differences are statistically high significant with the exception of the phosphate soluble in acid:

	Total *P*	Total P in sediment	Inorganic *P*	Organic P (soluble in acid)	Organic P (not soluble in acid)
Caries resistant	48,3	40,6	37,6	3,1	7,4 (mg/100 ml)
Caries active	29,3	26,2	24,3	2,1	3,1

IN DER Kariesforschung sind wenige Fragen so oft untersucht worden wie die, ob zwischen dem Phosphatgehalt des menschlichen Speichels und dem Auftreten von Karies ein Zusammenhang besteht. Trotz zahlreicher Untersuchungen (BECKS

*Wir danken der Deutschen Forschungsgemeinschaft für finanzielle Unterstützung.

und WAINWRIGHT, 1946; BRAWLEY und SEDWICK, 1940; EGGERS LURA, 1947; ENTIN und GEIKIN, 1928; KARSHAN, 1939; KNAPPWOST, 1952; MATHIS, 1934; STRÅLFORS, 1959; ERICSSON, 1949; u.a.) konnte die Frage nicht eindeutig beantwortet werden. Der Fortschritt unserer Erkenntnisse auf dem Gebiet der biochemischen Speicheluntersuchungen läßt heute drei Faktoren erkennen, die als Hauptursachen für die bisher uneinheitlichen Ergebnisse in Betracht kommen:

1. Mangelnde Standardisierung bei der Speichelprobenentnahme,
2. ungenaue Klassifizierung der Probanden in Kariesanfällige und Kariesresistente,
3. ungeeignete Methoden der Phosphatbestimmung.

Es ist Aufgabe dieser Arbeit, das Problem unter Berücksichtigung der obengenannten Punkte erneut zu bearbeiten.

MATERIAL

Die Versuchspersonen entleerten unmittelbar nach dem morgendlichen Erwachen 4 ml Speichel in ein graduiertes Glasröhrchen. Die Verarbeitung der Proben im Laboratorium begann sogleich um 8 Uhr morgens. Die Probanden waren angewiesen, die Proben bis zur Ablieferung kühl zu halten. Als Grundlage der Auswertung dienten 40 auf diese Art gewonnene Speichelproben.

METHODEN

In jeder Speichelprobe wurde bestimmt:

1. Sauerstoffverbrauch;
2. Gesamtphosphat;
3. Gesamtphosphat im enteiweißten Speichelüberstand;
4. Anorganisches Phosphat.

Aus den Werten für 2–4 wurde nach folgendem Schema der Gehalt der Proben an Phosphat errechnet:

Gesamtphosphat — anorganisches Phosphat = organisches Phosphat (gesamt)
Gesamtphosphat — Gesamtphosphat im enteiweißten Überstand = nicht säurelösliches organisches Phosphat
Gesamtphosphat im Überstand — anorganisches Phosphat = säurelösliches organisches Phosphat.

Die Messung des Sauerstoffverbrauchs wurde nach der von UMBREIT, BURRIS und STAUFFER (1957) beschriebenen Warburg-Manometertechnik vorgenommen.

Ansatz: 1,25 ml Veronalpuffer, pH 6,9
0,25 ml Speichel
1,00 ml H_2O
0,30 ml 10-prozentige NaOH (Zentralgefäß)

Die Bestimmung des Gesamtphosphates (Methode 2) erfolgte nach feuchter Veraschung mit einem Gemisch aus H_2SO_4 und $HClO_4$. Nach Enteiweißen der Probe mit $HClO_4$ und Zentrifugieren wurde im klaren Überstand das anorga-

nische Phosphat (Methode 4) und nach Veraschung das Gesamtphosphat im Überstand (Methode 3) bestimmt. Eine ausführliche Beschreibung aller Methoden findet sich bei AHRENS (1961).

ERGEBNISSE

Die Resultate dienten zur Beantwortung von drei Fragestellungen:

1. Besteht eine Beziehung zwischen Sauerstoffverbrauch und Phosphatgehalt?
2. Stehen die einzelnen Phosphatfraktionen untereinander in einem bestimmten Verhältis?
3. Wie groß sind die Unterschiede der einzelnen Phosphatfraktionen zwischen der Gruppe der Kariesanfälligen und der Gruppe der Kariesresistenten?

Zu 1

Die Werte jeder Phosphatfraktion aller Proben wurden in einem Koordinatensystem gegen den Sauerstoffverbrauch aufgetragen, wie es in Abb. 1 als Beispiel für anorganisches und organisches Phosphat gezeigt wird. Die statistische Auswertung erfolgte durch Aufstellen des Korrelations- und Regressionskoeffizienten

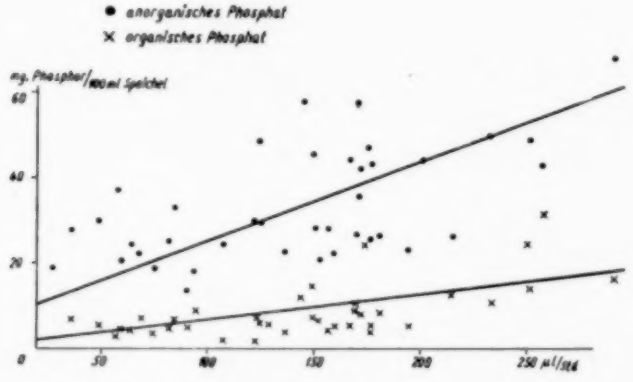


ABB. 1. Korrelation von anorganischem und organischem Phosphat mit der Sauerstoffaufnahme des menschlichen Speichels.

sowie durch Einzeichnen der daraus errechneten Regressionsgeraden in die graphische Darstellung. Es ist trotz der Streuung deutlich zu erkennen, daß mit steigendem Sauerstoffverbrauch auch die Werte für anorganisches und organisches Phosphat ansteigen. Die Regressionsgeraden als beste Schätzung der mittleren Beziehung zum Sauerstoffverbrauch verdeutlichen den Eindruck einer positiven Korrelation noch mehr. Die Korrelationsbilder für säurelösliches und nicht säurelösliches Phosphat sowie für Gesamtphosphat ähneln dem gezeigten Beispiel, so daß auf

Vol. 6
1961

ihre Darstellung verzichtet werden kann. Die genauen Werte der Korrelationskoeffizienten und ihre statistische Sicherung betrugen:

Korrelation des O_2-Verbrauchs mit:	r^*	Stat. Sicherung (%)
Gesamtphosphat	0,648	99,9
Anorganisches Phosphat	0,934	99,9
Organisches Phosphat	0,645	99,9
Nicht säurelösl. org. Phosphat	0,658	99,0
Säurelösl. org. Phosphat	0,331	90,0

*r gibt die Strenge einer Korrelation an. Beim Nichtvorhandensein ist $r = 0$ und liegt sonst zwischen $+1$ und -1. Die statistische Sicherung gibt hier die Wahrscheinlichkeit an, mit der sich r signifikant von 0 unterscheidet.

Es kann somit festgestellt werden, daß zwischen den Werten der untersuchten Phosphatfraktionen und dem Sauerstoffverbrauch im Speichel eine echte Korrelation besteht. Für säurelösliches organisches Phosphat betrug die statistische Sicherung nur 90 Prozent, so daß das Ergebnis in Bezug auf diese Fraktion nicht als ganz eindeutig gelten darf.

Zu 2

Die Versuchspersonen wurden nach dem klinischen Bild und der Sauerstoffaufnahme ihres Speichels in eine kariesanfällige und in eine kariesresistente Gruppe eingeteilt. Für jede Gruppe wurde der mittlere prozentuale Anteil des anorganischen und des organischen Phosphats am Gesamtphosphat errechnet (Abb. 2). Der In-

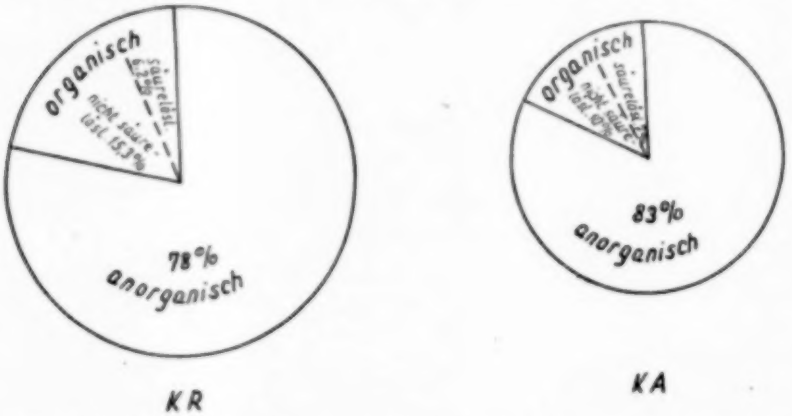


ABB. 2. Verhältnis zwischen anorganischen und organischen Phosphatfraktionen im Speichel von kariesresistenten und kariesanfälligen Personen. Der Inhalt der Kreisfläche entspricht dem durchschnittlichen Gesamtphosphatgehalt.

halt der Kreisfläche entspricht dem mittleren Gesamtphosphatgehalt jeder Gruppe. Man erkennt, daß Kariesanfällige einen verringerten Gesamtphosphatgehalt haben. Im relativen Verhältnis der anderen Fraktionen bestehen zwischen beiden Gruppen nur geringfügige Unterschiede. Es wurde mit dem Studentschen *t*-Test* geprüft, ob diese Unterschiede zufällige oder signifikante Abweichungen sind:

	Mittl. Differenz zwischen Gesamt-Phosphat u. anorganischem Phosphat	Mittl. Differenz zwischen Gesamtphosphat im Vollspeichel u. Gesamtphosphat im enteiweißten Überstand
KR	103,3	78,1
KA	50,5	31,4
t-Wert	2,467	2,167
Stat. Sicherung (%)	95	95

Die Unterschiede der Mittelwerte sind nicht signifikant. Man kann also folgern, daß die untersuchten Mittelwerte sich in beiden Personengruppen nur zufällig voneinander unterscheiden; mit anderen Worten: es besteht ein festes Verhältnis aller Phosphatfraktionen zueinander, und dieses Verhältnis ist bei Kariesanfälligen und bei Kariesresistenten gleich. Der verringerte Gesamtphosphatgehalt bei Kariesanfälligen kommt also dadurch zustande, daß alle Fraktionen gleichmäßig vermindert sind.

Zu 3

Die Mittelwerte der Gruppendurchschnitte aller Fraktionen wurden errechnet und ihre Differenzen mit dem Studentschen *t*-Test geprüft:

	Gesamt-P. (mg %)	Ges.-P. im Überstand (mg %)	anorg.-P. (mg %)	Gesamt (mg %)	Organisches Phosphat	
					säurelöslich (mg %)	Nicht säurelöslich (mg %)
KR	48,3 ±3,2	40,6 ±2,8	37,6 ±2,5	10,4 ±1,3	3,1 ±0,7	7,4 ±0,9
KA	29,3 ±1,9	26,2 ±1,8	24,3 ±1,8	5,1 ±0,6	2,1 ±0,4	3,1 ±0,6
t-Wert	3,93	3,93	3,52	2,68	0,97	3,02
Statistische Sicherung	99,9	99,73	99,73	99	—	99

*Alle statistischen Verfahren wurden KOLLER (1955) entnommen.

Vol. 6 1961

Es bestehen also in allen Fraktionen erhebliche Unterschiede zwischen beiden Gruppen (Abb. 3). Die Werte der kariesresistenten Gruppe sind stets höher.

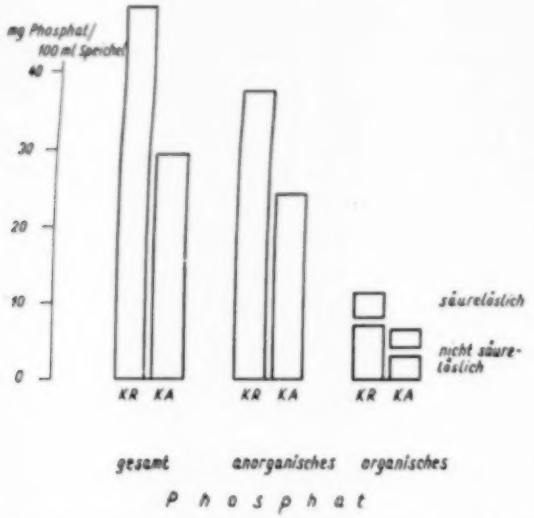


ABB. 3. Gegenüberstellung einiger Phosphatfraktionen im Speichel Kariesanfälliger und -resistenter.

Sie betragen in einigen Fällen fast das Doppelte der Kariesanfälligen. Mit Ausnahme des säurelöslichen organischen Phosphats sind alle Unterschiede statistisch gesichert.

DISKUSSION

Die vorliegende Arbeit erstrebt einen Vergleich zwischen Speichelphosphatgehalt und Kariesanfälligkeit. Die Schwierigkeit liegt—wie bei allen ähnlichen Arbeiten—in der exakten Bestimmung des Grades der Kariesanfälligkeit. Eine einmalige Erhebung des Gebißbefundes (z. B. die Aufstellung des DMF-Index) läßt bekanntermaßen nur bei großen Personengruppen, wie sie bei epidemiologischen Untersuchungen vorliegen, einen Hinweis auf die Kariesaktivität zu. Bei kleineren Gruppen ist eine vorausgehende Kontrollzeit von mindestens einem Jahr und ein Vergleich der Gebißbefunde vor und nach der Kontrollzeit zu fordern (BAUME, 1956).

Die vorliegenden Untersuchungen wurden während der Kontrollzeit des betreffenden Personenkreises unternommen. Da infolgedessen die klinische Kariesanfälligkeit noch nicht endgültig feststand, gingen wir, gestützt auf Arbeiten von BRAMSTEDT und Mitarbeitern (BRAMSTEDT und VONDERLINN, 1952; NAUJOKS und VONDERLINN, 1953; KRÖNCKE und NAUJOKS, 1956; KRÖNCKE, 1960) von der Voraussetzung aus, daß die Höhe des Sauerstoffverbrauchs eine gute Schätzung der Kariesdisposition bei Gruppenuntersuchungen darstellt. Da dieses Verfahren sich oft

bewährt hat und auch von anderer Seite Bestätigungen seiner Brauchbarkeit vorliegen (BURNETT, 1952; KOTHE und SCHÜTZMANNSKY, 1957), erschien seine Anwendung gerechtfertigt.

Die Tatsache, daß die gefundenen Phosphatwerte im Vergleich zu anderen Autoren sehr hoch liegen, wurde bereits an anderer Stelle (AHRENS, 1961) ausführlich diskutiert. Sie ist hauptsächlich auf die Verwendung des ersten morgendlichen Nüchternspeichels als Untersuchungsmaterial zurückzuführen.

Das vermehrte Auftreten von anorganischem Phosphat im Speichel Kariesresistenter wurde schon von anderen Autoren festgestellt (KARSHAN, KRASNOW und KREJCI 1931; KARSHAN 1939; ERICSSON, 1949). Nur waren die gefundenen Unterschiede nicht so deutlich, weil Tages- oder stimulierter Speichel verwendet wurde.

Über das organische Phosphat im menschlichen Speichel liegen bisher nur wenige Untersuchungen vor (EGGERS-LURA, 1949). Einige befassen sich mit bestimmten Verbindungen der organischen Fraktion (NAUJOKS *et al.*, 1958, AHRENS und RANKE, 1961). Die bisherigen Arbeiten stimmen mit der vorliegenden darin überein, daß eine Vermehrung der organischen Phosphatfraktion oder bestimmter, ihr zugehörender Verbindungen (z. B. der energiereichen Phosphate) im Speichel kariesresistenter Personen gefunden wurde. Diese Ergebnisse stehen im engen Zusammenhang mit der bei Kariesresistenten ebenfalls erhöhten Sauerstoffaufnahme, da organische Phosphate am Ablauf des Kohlenhydratabbaus und damit am Ausmaß des Sauerstoffverbrauchs beteiligt sind (BRAMSTEDT *et al.*, 1954).

LITERATUR

AHRENS, G. 1961. *Zur Problematik von Phosphatbestimmungen im menschlichen Speichel.* Unveröffentlichte Versuche.

AHRENS, G. und RANKE, B. 1961. Energiereiche Phosphate im menschlichen Speichel. *Dtsch. zahnärztl. Z.* **16**, 196–199.

BAUME, L. J. 1956. Vorschläge zur Vereinheitlichung kariesstatistischer Befunderhebung und Auswertung. *Dtsch. zahnärztl. Z.* **11**, 355–360.

BECKS, H. und WAINWRIGHT, W. W. 1946: Relationship of total calcium and inorganic phosphorus of resting saliva. *J. dent. Res.* **25**, 267–274.

BRAMSTEDT, F., KRÖNCKE, A., NAUJOKS, R. und VONDERLINN, R. 1954. Die Verbrennung organischer Substrate im Speichel kariesresistenter und kariesanfälliger Personen. *Dtsch. zahnärztl. Z.* **9**, 782–791.

BRAMSTEDT, F. und VONDERLINN, R. 1952. Die Sauerstoffaufnahme des Speichels und ihre Beziehung zur Zahnkaries. *Zahnärztl. Welt* **7**, 505–509.

BRAWLEY, R. E. und SEDWICK, J. H. 1940. Studies concerning the oral cavity and saliva—V. phosphorus. *J. dent Res.* **19**, 315–319.

BURNETT, G. W. 1952. The respiration rate of human saliva. *J. dent. Res.* **31**, 468–469.

EGGERS-LURA, H. 1947. Investigations on the salivary phosphates and phosphatases. *J. dent. Res.* **26**, 203–224.

EGGERS-LURA, H. 1949. Untersuchungen über die Remineralisation kariöser Zähne. *Schweiz. Mschr. Zahnheilk.* **59**, 181–189.

ENTIN, D. A. und GEIKIN, M. K. 1928. Über den Phosphatgehalt im Speichel. *Dtsch. Mschr. Zahnheilk.* **46**, 867–885.

ERICSSON, Y. 1949. *Enamel-apatite Solubility.* Stockholm.

KARSHAN, M. 1939. Factors in saliva correlated with dental caries. *J. dent. Res.* **18**, 395–407

KARSHAN, M., KRASNOW, F. und KREJCI, L. E. 1931. A study of blood and saliva in relation to immunity and susceptibility to dental caries. *J. dent. Res.* **11**, 573–589.

KNAPPWOST, A. 1952. Grundlage der Resistenztheorie der Karies. *Dtsch. zahnärztl. Z.* **7**, 670–680.

KOLLER, S. 1955. Statistische Auswertung der Versuchsergebnisse in: HOPPE-SEYLER, THIER-FELDER, *Handbuch der Physiologisch- und Pathologisch-Chemischen Analyse*, Berlin. 10.Aufl.

KOTHE, J. und SCHIITZMANNSKY, G. 1957. Vergleichende Untersuchungen zwischen Kariesanfälligkeit und Sauerstoffverbrauch des Speichels und ihre Verwendbarkeit als Kariestest. *Dtsch. Zahn- Mund- u. Kieferheilk.* **27**, 347–352.

KRÖNCKE, A. 1960. Ergebnisse und Probleme biochemischer Speichelforschung. *Dtsch. Zahnärztabl.* **17**, 1–5.

KRÖNCKE, A. und NAUJOKS, R. 1956. Dental caries susceptibility tests and their significance in dental practice. *Int. Dent. J., Lond.* **6**, 174–188.

MATHIS, H. 1934. Beiträge zur Chemie des Speichels. *Z. Stomat.* **32**, 1188–1209.

NAUJOKS, R., BRAMSTEDT, F., KRÖNCKE, A. und MARG, H. 1958. Einfluß niedriger Fluorkonzentrationen auf die energiereichen Phosphate des Speichels. *J. Dent. Belge* **6**, 403–416.

NAUJOKS, R. und VONDERLINN, R. 1953. Klinische Überprüfung der Möglichkeiten zur Entwicklung eines biochemischen Kariestestes auf der Grundlage des Sauerstoffverbrauches des menschlichen Speichels. *Zahnärztl. Rdsch.* **62**, 1–4.

STRÅLFORS, A. 1959. The effect of calcium and phosphate ions on the rate of enamel dissolution. *Odont. Revy* **10**, 367–378.

UMBREIT, W. W., BURRIS, R. H. und STAUFFER, J. F. 1957. *Manometric Techniques.* Burgess, Minneapolis.

Vol.
196

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 249–259, 1961. Pergamon Press Ltd.

SOME PHYSICO-CHEMICAL PROPERTIES OF THE CARIOUS AND NON-CARIOUS SALIVA

D. HATTYASY, I. SZABÓ and K. TÓTH

Research Department, University Clinic, and Hospital for Oral and Dental Diseases, University Medical School, Szeged, Hungary

Vol. 6
1961

Abstract—The aim of our investigation was to determine whether there is a correlation between the rate of salivation, the clinical picture, the acid producing power, the pH value, the *pK* value, the redox potential and the oxygen consumption of the saliva of active carious, of arrested carious, and of non-carious juveniles. 63 boys between the ages of 7 and 14 were examined clinically and using the Snyder test; we selected three groups, each of ten test persons for tests concerning acid formation (Sn) and caries:

(1) Sn negative or weakly positive who had good teeth;
(2) Sn medium positive, mostly with arrested caries;
(3) Sn strongly positive, the majority with active multiple caries.

Results: *In vitro*, the pH of the saliva moves to the alkaline side; however it shows a certain amount of stability. In avg. the cases of groups (1) and (2) have 0·3 pH higher values; in the three groups the pH and p*K* values show strikingly similar differences; for the groups (1) and (2) the pH values in avg. are almost identical. There was not much difference in the oxygen consumption of the three groups but there was a tendency towards low values even in the most active clinical cases. Low salivation speed and relatively low oxygen consumption were found for a number of non-carious and active saliva specimens; the difference however lies in the fact that the latter were strongly Sn positive. The potential determinations do not seem to show a correlation in our material.

Selected cases of group (1) [compared with selected cases of group (3)] have in avg. 0.54 higher intraoral pH and 2,085 higher p*K* values (see Tab. 2,3,4).

Zusammenfassung—Unser Ziel war das Bestehen einer Korrelation zu untersuchen zwischen der Geschwindigkeit der Speichelabsonderung, dem klinischen Bild, dem Säurebildungsvermögen, dem pH-Wert, dem p*K*-Wert, dem Redoxpotential und des O_2-Verbrauches des Speichels aktiv kariöser, passiv kariöser (arrested) und kariesfreier Jugendlicher. Auswahl nach dem klin. Bild und Snyder-Test aus 63 Jungen zw. 7–14 Jahren; es wurden 3 Gruppen zu je 10 Probanden gebildet, bezüglich der Säurebildung (Sn) und Karies: (1) Sn neg. u. schwach pos., gute Zähne; (2) Sn mittelstark pos., meist mit passiver Karies: (3) Sn stark pos., überwiegend mit aktiven multiplen Kariesherden.

Resultate: *In vitro* verschiebt sich das pH des Speichels n. d. alkalischen Seite, zeigt aber eine gewisse Konstanz. Die Fälle d. Gruppen (1) u. (2) haben im D einen um 0,3 pH grösseren Wert; die pH u. p*K*-Werte zeigen auffallend ähnliche Differenzen in den 3 Gruppen; die pH-Werte der Gruppen (1) u. (2) sind im D fast identisch. Der O_2-Verbrauch zeigte b. d. Gruppen keine wesentliche Differenzen, aber eine Tendenz zu niedrigen Werten eben b. d. klinisch am meisten aktiven Fällen. Geringe Geschwindigkeit in der Speichelabsonderung und rel. geringer O_2-Verbrauch waren für eine Anzahl der kariesfreien und aktiven Speichel zu finden, mit dem

Unterschied jedoch, dass letztere stark Sn positiv waren. Die Potentialbestimmungen scheinen an unserem Material keine Korrelation zu zeigen.

Selektierte Fälle der Gruppe (1) zeigen gegenüber solchen der Gruppe (3) im D um 0,54 höhere intraorale pH-Werte und um 2,085 hörere p*K*-Werte (s. Tab. 2,3,4).

THE DIFFERENTED results obtained by different investigators concerning saliva pH (2, 8, 14, 30), buffering capacity (5, 11, 15, 16, 17, 19, 20, 24, 38, 39, 42), acid production, redox potential (eH) (6, 9, 10, 19, 21, 41), rate of flow of saliva and oxygen-consumption (1, 3, 4, 7, 12, 29, 35) not only in the carious values but in the physiological values as well prompted us to investigate these factors in a selected group of young human males.

We wished to obtain data on the same individuals concerning these different factors, viz. mechanisms, and to be able contribute some new results to those so far obtained by the said authors. We were anxious to determine which test (7, 31, 25, 38) or tests could be used most effectively for assesing caries susceptibility.

Initially young individuals were chosen living in a relatively constant environment and social background; from this material seemingly good average results (22, 23, 35, 37) have been already obtained.

We selected 15 boys from 6 to 13 years old with no carious teeth and 24 boys with one or more lost or decayed teeth between 6 and 14 years old. All these individuals were living in a children's home, in a sort of college (33, 34). As we will show later we had to look for another group of boys with bad teeth because this college "carious" group showed itself very similar to the group with intact teeth. This later group consisted of 23 boys, of 10–12 years of age, with one or more missing and decayed teeth and were pupils of a public school in the city (32).

METHODS

1. *Caries: clinical and X-ray.* Classified as caries free were subjects with no caries at all and no disintegrated fissures who showed no more than a + + Snyder-reaction after 48 hr repeated tests (12, 25, 26, 27, 28). The carious-group contains cases with six or more DMF teeth and a + + Snyder or more after 24 hr.

2. *Collection and rate of flow of saliva.* Whole saliva was collected before breakfast without cleaning the teeth. Most of the boys spat in a marked glass-container (groups I, II, III); in some cases we obtained it with suction (groups IV and V) and noted the quantity after 5, 10 and 20 min; we used no additional stimulation. The saliva samples were collected partly in a nearby school (groups I and II) and could be used in half an hour, partly in the laboratory (group III), and as a complementary part of these investigations there were two small groups (groups IV and V) from which the saliva samples were taken individually in the laboratory and employed instantly.

3. *Acid production.* Snyder-tests as modified by us (31) were carried out and repeated on two or three successive days. Readings were obtained after 24 and 48 hr. Colour intensity with 0–4 x-marks.

4. *pH-determinations.* These were made with the Danish universal pH-meter 22, Copenhagen, partly *in vitro* after allowing the specimen to stand for different lengths of time, partly intraorally with a glass-electrode.

5. *Buffering-capacity.* (p*K*) with Ericsson's (11) method clinical test I. Greater buffering capacity means greater values and a minor difference between pH and p*K*.

4. *Redox potential-determinations.* These were made with the platinum-electrode against the calomel-electrode. We have used three electrodes. The readings were generally taken after 2 min but in some cases after 1, 2, 5, 10, 20 and 30 min. The electrodes were cleaned after each determination in chromic-sulphuric acid. Before use the electrodes were standardized in a $Fe^{II} - Fe^{III}$ containing solution.

7. *Oxygen consumption.* (W). This was determined in the Warburg-manometers after the instructions given by UMBREIT, BURRIS and STAUFFER, *Manometric Technique and Tissue Metabolism* (40). Parallels and thermic-control-manometer were always used. Differing parallels were discarded. In the experimental container were mixed saliva, 1 ml; phosphate buffer, pH 7·2, 2 ml; 10% NaOH, 0·2 ml. In the control-container distilled water replaced saliva.

Vol. 6
1961

PROCEDURE AND RESULTS

From the subjects originally examined were selected typical individuals with no carious and with active carious teeth, ten in each group.

We found in the above mentioned college-type school 15 boys with good teeth (group Ia), of these 10 boys showed a very weak Snyder-reaction and these were selected for the further tests (group Ib). Similarly, but from the group of 24 boys with carious teeth (group IIa), were selected 10 such boys showing a strong positive Snyder–reaction (group IIb). We found in this group IIa, 10 subjects who gave repeatedly only a slightly positive Snyder-test; the remaining 4 showed variable results to the Snyder test.

In these two groups (Ib and IIb) the results of the pH, p*K*, and W-test were very similar (Tables Ia and b). Clinical examination usually suggested that the carious lesions in group IIb were arresting as they were.

We looked now for more genuine carious-cases which we found in the city-public school, where out of 23 seemingly active cases (group IIIa) we selected the ten most Sn-positive cases (group IIIb).

In Tables Ia, b, c, we also indicate for the three groups (Ib, IIb, IIIb) the corresponding W-average-values after 1 hr from three or four determinations carried out. usually on consecutive days. The W-values differ greatly due to the actual saliva spitting technique of the individual. Therefore it seemed necessary to discard values with extremely low or high differences. Extremely low values occur when the sublingually accumulated saliva is allowed to drop out. Such saliva has a very low bacteria content. High values are found when with the suction during spitting a great quantity of bacteria is dislodged into the sputum from around the teeth.

TABLE Ia. GROUP Ib. SOUND TEETH—COLLEGE

No.	W*	pH†	p*K*‡	Diff. pH—p*K*
3	51	7·63	5·55	2·08
11	136	7·76	6·63	1·13
12	105	7·41	5·18	2·23
5	345	7·45	6·01	1·44
9	126	7·13	5·90	1·23
14	87	7·56	5·86	1·70
2	138	7·65	5·95	1·70
7	117	7·60	5·20	2·40
8	192	7·35	6·32	1·03
10	196	6·91	5·13	1·78
Avg.	149	7·44	5·77	1·67
Min. val.	51	6·91	5·13	1·13
Max. val.	345	7·76	6·63	2·23

*Oxygen consumption Warburg (avg.) 1hr values of three determinations.

†*In vitro* after 30 min (avg.)

‡After the pH determination.

TABLE Ib. GROUP IIb. BAD TEETH—COLLEGE

No.	W*	pH†	p*K*‡	Diff. pH—p*K*
19	211	7·25	5·53	1·72
26	248	7·41	5·86	1·55
29	142	8·06	6·85	1·21
33	175	7·06	5·06	2·00
34	119	6·60	4·55	2·05
39	146	7·33	5·20	2·13
18	183	7·68	6·56	1·12
31	142	7·83	6·23	1·60
35	74	7·55	5·88	1·67
40	58	7·38	6·41	0·97
Avg.	150	7·41	5·81	1·60
Min. val.	58	6·60	4·55	1·12
Max. val.	248	8·06	6·85	2·13

*Oxygen consumption Warburg (avg.) 1hr values of three determinations.

†*In vitro* after 30 min (avg.)

‡After the pG determination.

The results in Tables 1a, b, c, show a great similarity in the averages and a great diversity in the individual values. We got the impression from these results that a further selection of the cases would be advisable.

TABLE 1c. GROUP IIIb. BAD TEETH—PUBLIC SCHOOL

No.	W*	pH†	p*K*‡	Diff. pH—p*K*
41	105	7·68	5·88	1·80
44	82	6·81	4·66	2·15
45	208	7·48	5·25	2·23
51	95	7·03	5·48	1·15
55	138	6·88	5·28	1·60
56	148	7·33	6·18	1·15
58	133	7·08	4·83	2·25
59	92	7·13	5·26	1·87
60	149	7·20	5·83	1·37
63	95	7·10	5·81	1·29
Avg.	124	7·17	5·45	1·73
Min. val.	82	6·81	4·66	1·15
Max. val.	208	7·68	6·18	2·25

*Oxygen consumption Warburg (avg.) 1hr values of three determinations.

†*In vitro* after 30 min (avg.)

‡After the pH determination.

Therefore we decided to re-investigate cases No. 3, 9, 11, 12, 14 from Table 1a and cases No. 4, 19, 15, 18, 51 from Table 1c. These cases were selected from Table 1a on account of their low oxygen consumption and from Table 1c on account of their relatively higher oxygen consumption, and on account of the presence of one or more soft, light coloured carious lesions clinically. Table 2 shows the findings for the non-carious group and Table 3 those of the carious group. (The averages of Table 2 and Table 3 are compared in Table 4).

In these two groups the averages differ but the cases inside the groups show a much more greater similarity to each other than those in Tables 1a, b, c. In Table 4 it is seen that the intraoral pH in the caries free group is 0·54 pH higher than in the caries group and is near neutrality (pH 6·91).

The second significant finding is the much greater buffering capacity of the cariesfree saliva.

Oxygen consumption, as seen in Tables 1a, b, c, shows no definite trends (In the discussion we will come back to this point).

TABLE 2. SELECTED CARIES-FREE CASES—SALIVA

No.	Age (year)	First examination						Second examination					
		Date	pH io.*	pH 60 min ‡	Diff. pH io.—60 min	p*K*	Diff. pH io.—p*K*	pH io.	pH 60 min	Diff. pH io.—60 min	p*K*	Diff. pH io.—p*K*	Date
3	9	V 22	6·75	7·80	+1·05	6·25	−0·50	6·85	7·55	+0·70	6·05	−0·80	V 23
9	12	V 22	6·70	7·55	+0·85	5·50	−1·20	6·55	7·60	+1·05	5·30	−1·25	V 23
11	12	V 17	6·90	—	—	6·55	−0·35	7·05	7·85	+0·80	6·20	−0·85	V 23
12	12	V 17	7·20	—	—	6·30	−0·90	7·05	7·75	+0·70	6·30	−0·75	V 18
14	14‡	V 23	7·00	7·60	+0·60	6·10	−0·90	7·10	8·05	+0·95	6·20	+0·90	V 24
	11·8	Avg.	6·91	7·65	+0·74	6·14	−0·77	6·92	7·76	+0·84	6·01	−0·91	Avg.
	9	Min. val.	6·70	7·55	+0·60	6·10	−0·35	6·55	7·55	+0·70	6·05	−0·75	Min. val.
	14	Max. val.	7·20	7·80	+1·05	6·55	−1·20	7·10	8·05	+1·05	6·30	−1·25	Max. val.

* io. = intraoral.

† *in vitro* 60 min after collecting.

‡ After breakfast.

TABLE 3. SELECTED ACTIVE CARIOUS CASES—SALIVA

No.	Age (years)	First examination						Second examination					
		Date	pH io.	pH 60 min	Diff. pH io.—60 min	p*K*	Diff. pH io.—p*K*	pH io.	pH 60 min	Diff. pH io.—60 min	p*K*	Diff. pH io.—p*K*	Date
44	13	V 24	6·55	7·15	+0·60	3·70	−2·85	6·55	7·25	+0·70	3·50	−2·95	V 25
51	13	V 24	6·30	7·30	+1·00	4·20	−2·10	6·45	7·20	+0·75	4·55	−1·90	V 25
55	13	V 26	6·45	7·25	+0·80	4·45	−2·00	6·45	7·40	+0·95	5·10	−1·35	V 27
58	13	V 26	6·50	7·30	+0·80	4·80	−1·70	6·25	7·30	+1·05	3·25	−3·00	V 28
59	12	V 26	6·10	7·20	+1·10	3·15	−2·95	6·15	7·25	+1·10	3·20	−2·95	V 27
		Avg.	6·38	7·22	+0·86	4·06	−2·32	6·37	7·28	+0·91	3·92	−2·43	Avg.
		Min. val.	6·10	7·15	+0·60	3·15	−1·70	6·15	7·20	+0·70	3·20	−1·35	Min. val.
		Max. val.	6·55	7·30	+1·10	4·80	−2·95	6·55	7·40	+1·10	5·10	−3·00	Max. val.

Vol. 6
1961

TABLE 4. AVERAGES AND DIFFERENCES OF TABLES 2 AND 3

	Avg. of two exam. caries neg. cases (from Table 2)	Avg. of two exam. caries pos. cases (from Tabl. 3)	Diff. caries neg.—caries pos. cases
pH io.	6·915	6·375	+0·540
pH 60 min	7·705	7·250	+0·455
Diff. pH io.—pH 60 min	+0·790	+0·885	−0·095
p*K*	6·075	3·990	+2·085
Diff. pH io.— p*K*	−0·840	−2·375	+1·535

TABLE 5. SALIVA POTENTIALS

No.	College-boys Caries-free*	No.	College-boys Carious teeth†	No.	Public-school boys Carious teeth‡
(3)	290—325 360	19	305—360 410	41	230—265 300
(11)	195—320 340	26	360—380 400	[44]	160—230 300
(12)	340—370 400	29	325—370 415	45	250—255 260
5	235—245 255	33	315—320 325	[51]	310—325 340
(9)	305—360 420	34	410—460 510	[55]	270—310 350
(14)	265—320 370	39	250—265 280	56	310—330 350
2	250—260 270	18	260—275 290	[58]	350—375 400
7	270—290 415	31	345—380 420	[59]	250—285 320
8	290—350 400	35	445—450 455	60	380—400 420
10	250—280 425	40	295—330 370	63	350—370 400
Avg.	312		359		314

[] Selected active carious cases as in Table 3.
() Selected caries-free cases as in Table 2.
* As in Table 1a.
† As in Table 1b.
‡ As in Table 1c.

The redox potential of the saliva is also a problem investigated by many authors (6, 9, 10, 19, 21, 41). We have, as already mentioned tried to determine the saliva-potential with the platinum-electrode against calomel-electrode. We have great doubts if this is at all possible because there is no constant value obtainable—certainly not until after a longer time (30 min or more). The values in Table 5 are typical findings from many estimations using different electrodes and are 2 min averages.

TABLE 6. CHANGE OF SALIVA-POTENTIAL

Time (min)	First measurement	Second measurement*	Third measurement
Immersion	540	560	555
1	350	385	490
2	295	325	340
3	265	295	300
4	250	270	285

* Cleaning was carried out between first and second, and third measurements.

There is seemingly no difference between the three groups. It is probable that on the surface of the platinum-electrode a process which leads to the diminishing potentials begins immediately after its immersion. If we assess the same saliva-specimen repeatedly with the same cleaned platinum-electrode, each time we obtain the same initial-potential which diminishes rapidly as in the given example (Table 6).

DISCUSSION

This investigation sometimes supports and sometimes refutes methods which have been used for comparing the properties of saliva in caries-free and caries-active mouths.

Of great importance in our opinion is the selection of subjects. This seems to be the surest method clinically. In other words, where there are many progressive carious lesions different results are to be expected as shown in Tables 2 and 3. We used three methods of selection: the first clinically (DMF-numbers and X-ray), then with the Snyder-test, and the third between caries-free cases with the low oxygen consumption and/or cases with strong positive Snyder-test, high oxygen consumption and last, but not least the presence of clinically active carious lesions. Had we not followed this way the outstanding values would have been swallowed up by the averages as can be seen in Tables 1a, b, c.

This leads to the question of the methods used. The pH of the saliva changes extraorally, but not always in the same way (Table 7).

The difference between the intraoral and the 30 min readings varies between 0·55 and 0·90 pH. This value is therefore doubtful. The intraoral pH on the other hand in consecutive determinations is very closely the same.

For determination the buffer-capacity the ERICSSON method (11) seems to be good and can be trusted.

The Warburg-method for the oxygen consumption (W) is theoretically and practically very good if, and this is a big if, you put uniform material in the bottles. Some samples when the sputum could not be fully homogenized, showed parallels with different results. By the saliva-W arises another, and this is the greater, difficulty

TABLE 7. CHANGE OF THE pH OF THE SALIVA

No. / Time	3	9	11	14	44	51	58
io.	675	670	705	700	655	635	650
10 min	735	705	755	735	705	720	695
30 min	755	740	770	755	710	725	715
60 min	780	755	785	760	715	730	730

Note:

3, 9, 11, 4 are selected caries-free cases (see Table 2)

44, 51, 58 are selected carious cases (see Table 3). — io : intraoral determination.

10, 30 and 60 min after collecting the saliva; determined *in vitro* at room temperature.

that you can hardly get the same material from the mouth. The simpler technique cannot be trusted and there are no others. The W-value depends on the type and number of the micro-organism in the sample. TOTH (36) showed this very clearly and in this study we have also seen that a sample got with suction or after centrifugation gives a very low W-value. The saliva sample-getting method should be improved, before these results can be regarded seriously. This easily explains why so many contradictory results have been reported (1, 3, 4, 7, 13, 29, 35). We do not say that the W-method cannot be employed; it is valuable only when results are examined critically. We could use it only on selected cases, in which we obtained repeatedly the same results with no more than $\pm$ 50 per cent difference. The lower values are suggestive of few bacteria in the sample and vice versa.

The determination of the redox-potential is, as mentioned above, the most difficult problem. This should be both theoretically and practically further investigated. We were unable to find correlation between caries-active and caries-free saliva and the resulting values. Notwithstanding, the problem is interesting and should be further investigated. We have already some findings which are going to be published elsewhere in the near future.

RESULTS

The results, viz. the test-methods we have used, are in our opinion to be divided into two groups:

Methods which give some orientation and methods which give some assurance. Assurance that the carious process seems to be not only a localized lesion but works through the metabolism and so through the function of the salivary glands.

In the first group is included the Snyder-test and the oxygen-consumption. The former shows the intensity of the acid production by the oral microorganism, if the sample is taken in the right way; the latter one, number and oxygen-need of the oral microbes, again, if the sample is taken in the right way. By correct collecting — we can not say how correct collecting should be made — high values seem to indicate caries-susceptibility. But we are not sure if this is true.

In the second group there are the methods for the determination of the saliva-pH and buffer-capacity of the saliva. These two methods gave, in our study by right selection, results of great significance (Tables 2, 3 and 4). pH-determinations should be made in our opinion with a glass-electrode intraorally and eventually *in vitro* with different reading times. Values lower than pH 6·7 seem to be accompanied with caries. In our caries-free cases (Table 2) the average pH, 6·91, was shown very strongly. This can be said also respectively for the caries-active cases with their average of pH 6·38. If the cases are not cautiously selected then transitional values are obtained that may easily lead to false conclusions and wipe out the essential facts.

We should not forget that in the earlier literature the essential facts were clearly seen by PICKERILL (20a) and KARSHAN (15a).

REFERENCES

1. BRAMSTEDT, F., KRÖNCKE, A., NAUJOKS, R. and VONDERLINN, R. 1954. *Dtsch. zahnärztl. Z.* **9**, 782.
2. BRAWLEJ, R. E. 1935. *J. dent. Res.* **15**, 55.
3. BURNETT, G. W. 1952. *J. dent. Res.* **31**, 468.
4. CALANDRA, J. G. and ADAMS, E. C. 1951. *J. dents. Res.* **30**, 229.
5. DEWAR, M. R. 1950. *J. dent. Aust.* **22**, 24.
6. EGGERS-LURA, H. 1955. *J. dent. Res.* **34**, 752,
7. EGGERS-LURA, H. 1956. *Acta odont. scand.* **13**, 257.
8. EISENBRANDT, L. I. 1943. *J. dent. Res.* 22, 147.
9. EISENBRANDT, L. I. 1944. *J. dent. Res.* **23**, 313.
10. EISENBRANDT, L. I. 1945. *J. dent. Res.* **24**, 247.
11. ERICSSON, Y. 1959. *Acta odont. scand.* **17**, 131.
12. HARTMANN, F. W. 1941. *J. Amer. dent. Ass.* **28**, 44.
13. HARTLES, R. L. and WASDELL, M. R. 1955. *Brit. dent. J.* **98**, 77.
14. HUBBEL, R. B. 1933. *Amer. J. Physiol.* **105**, 436.
15. KARSHAN, M., KRASNOW, F. and KREJCI, L. 1931. *J. dent. Res.* **11**, 573.
15a. KARSHAN, M. 1936. *J. dent. Res.* **15**, 383.
16. KRASSE, B. and GUSTAFSON, B. 1955. *J. Amer. dent. Ass.* **56**, 50.

17. KRASNOW, F. 1932. *J. dent. Res.* **12**, 530.
18. LILIENTHAL, B. 1955. *Oral Surg.* **8**, 828.
19. MASAO ONOSI and WATURU KONDO. 1960. *J. dent. Res.* **39**, 433.
20. MURACCIOLE, J. C. 1955. *J. dent. Res.* **34**, 387.
20a. PICKERILL, H. P. 1912. *The Prevention of Dental Caries and Oral Sepsis.* Bailliere, Tindall Cox, London.
21. RICHARDSON, R. L. 1958. *J. dent. Res.* **37**1 697.
22. SZABÓ, I. and TÓTH, K. 1961. *Acta physiol., Acad. sci. hung.* In press.
23. SSABÓ, I. and TÓTH, K. 1961. *Acta physiol., Acad. sci. hung.* In press.
24. SELLMAN, R. M. 1950. *Acta odont. scand.* **8**, 244.
25. SNYDER, M. 1940. *J. dent. Res.* **19**, 349.
26. SNYDER, M. 1941. *J. Amer. dent. Ass.* **28**, 44.
27. SNYDER, M. 1947. *J. Amer. dent. Ass.* **29**, 2001.
28. SNYDER, M. 1942. *J. Amer. dent. Ass.* **42**, 400.
29. STRALFORS, A. 1956. *Acta odont. scand.* **14**, 153.
30. SCHMIDT-NIELSEN, B. 1946. *Acta Physiol. Scand.* **11**, 104.
31. TÓTH, K. and TÖRTELI, Á. 1955. *Fogorv. Szle* **48**, 73.
32. TÓTH, K., PÓNYI. S. and MOLDOVAY, F. 1957. *Fogorv. Szle* **50**, 96.
33. TÓTH, K. 1957. *Fogorv. Szle* **50**, 361.
34. TÓTH, K. and HATTYASY, D. 1959. *Acta med. Acad. sci. hung.* **14**, 141.
35. TÓTH, K. 1960. *Acta med. Acad. sci. hung.* **16**, 279.
36. TÓTH, K. 1960. *Acta med. Acad. sci. hung.* **16**, 319.
37. TÓTH, K. 1960. *Fogorv. Szle* **53**, 197.
38. TURNER, N. C., SCRIBNER, J. H. and BELL, J. 1954. *J. dent. Res.* **33**, 55.
39. TURNER, N. C. and ANDERS, J. T. 1956. *J. dent. Res.* **35**, 385.
40. UMBREIT, W., BURRIS, R. H. and STAUFFER, I. F. 1951. *Manometric Techniques and Tissue Metabolism.* Burgess, Minneapolis.
41. WESSINGER, G. 1941. *J. dent. Res.* **20**, 123.
42. WHITE, J. and BUNTING, R. 1936. *Amer. J. Physiol.* **117**, 529.

Vol. 6
1961

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 260–265. 1961. Pergamon Press Ltd.

FLUORIDATED CHEWING GUM AND TOOTHPASTE STUDIES WITH ^{18}F

R. DUCKWORTH and R. D. EMSLIE

Departments of Dental Medicine and Preventive Dentistry, Guy's Hospital, London S.E.1, England

INTRODUCTION

TOPICAL applications of fluoride solutions to the teeth have been shown to be of value in caries control. The procedure, however, is time consuming and impracticable for large numbers of children. The addition of stannous fluoride to toothpaste appears to reduce caries activity (PEFFLEY and MUHLER, 1960), especially when brushing is supervised. Tooth brushing in children is often inadeaquate; therefore a method of fluoride application which could and would be used by children themselves might be more effective.

Chewing gum as a vehicle for the administration of fluoride has been described by EMSLIE, VEALL and DUCKWORTH (1961). A summary of this work is presented here, together with data on the uptake by teeth of fluoride from fluoridated toothpaste.

MATERIALS AND METHODS

It seemed desirable that the chewing gum used should have a low calcium content and be free of known cariogenic agents. The formula of the chewing gum is:

	Per cent
Special chewing gum base	28
Staybelite Ester N/10	18
Sorbitol	24
Potato starch	28
Saccharine	0·3
Vanilla	0·15
Ol. menth. pip.	1
Ol. menth. virid.	0·55
	100

The special base is a synthetic, sugar free, low calcium material and staybelite ester is a resin used in many proprietary chewing gums. Sorbitol, which has been shown by KLAPPER and VOLKER (1954) to have little or no cariogenic activity, and saccharine were the sweetening agents. Potato starch was used as the filler instead of a calcium containing compound. Conventional flavouring agents were employed. Sodium

or stannous fluoride (0·1 mg F/g of gum) was added. The final calcium content of this mixture was 3 mg/g.

In view of the reported enhanced effect of acidulated fluoride solutions compared with aqueous solutions, this formula was modified, to test the effect of lowered pH, by adding citric and phosphoric acids. The influence of calcium content was tested by adding calcium carbonate and by replacing the filler by dicalcium hydrogen phosphate.

The experimental toothpaste contained 1 mg of F/g of paste: ten times the concentration of F in the chewing gum.

Both chewing gum and toothpaste were labelled with ^{18}F immediately before use. Gum and paste contained 1–2 μc per gram at the time of use. A well-type scintillation counter was used for radioactivity measurements.

In chewing experiments the initial radioactivity of a piece of gum was counted. The gum was then chewed for 1 min, the activity redetermined and expressed as a percentage of the original activity. This was repeated for a total chewing time of at least 10 min.

Uptake of fluoride was studied in children or young adults from whom sound teeth were to be extracted for orthodontic reasons. A weighed piece of ^{18}F labelled fluoridated chewing gum (approximately 1 g)) was chewed for 10 min immediately before extraction of the teeth. In the toothpaste studies the tooth to be extracted and the adjacent teeth were brushed for 1 min with a known weight (0·5–1 g) of ^{18}F labelled toothpaste. Before counting, extracted teeth were scrubbed with soap and water for 5 min to remove surface organic material which had absorbed ^{18}F.

To allow calculation of fluoride uptake by the teeth, standards were prepared by counting the activity of accurately weighed samples of gum or toothpaste.

RESULTS

Release of fluoride from chewing gum

Fig. 1 shows the pattern of release of ^{18}F labelled chewing gum containing either sodium fluoride or stannous fluoride. In each case the curve can be defined by the sum of two exponentials. Chewing the gum for 10 min released 80–90 per cent of the ^{18}F. This release was rather more rapid from gum containing stannous fluoride.

The addition of 2% citric acid to the standard formula caused up to 80 per cent of the fluoride to be retained in the gum after 10 min chewing (Fig. 2). Phosphoric and citric acids (0·5%) had little effect.

The release of fluoride was not materially altered when 5% calcium carbonate was added to the gum. Substitution of the potato starch filler by dicalcium hydrogen phosphate reduced the release of ^{18}F by a half (Fig. 2).

Vol. 6
1961

Uptake of ^{18}F *by teeth from chewing gum.*

This was calculated in terms of the uptake per 0·1 mg F in the gum, that is to say, the observed uptake is corrected for 100 per cent extraction of fluoride. From the sodium fluoride gum there was an uptake of ^{18}F corresponding to 9–60 mμg F.

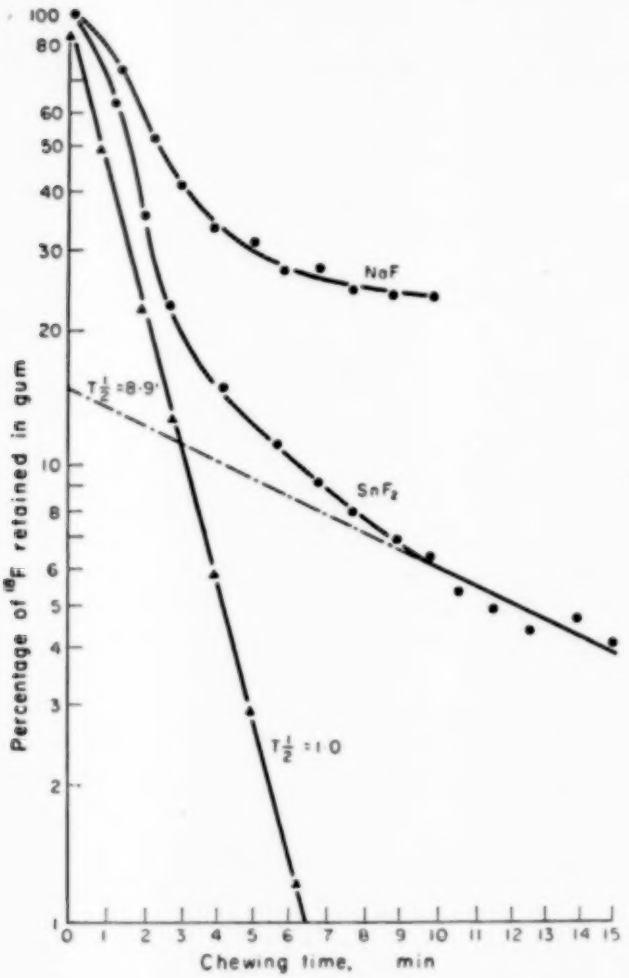


FIG. 1. Release of ^{18}F from chewing gum containing either sodium fluoride or stannous fluoride. The straight lines represent the two exponentials which define the curve of isotope release from the stannous fluoride gum. $T\frac{1}{2}$ indicates the half period of each exponential.

When 2% citric acid was added to sodium fluoride gum the uptake was increased to 75–172 mμg. The uptake of fluoride by sound premolars, from stannous fluoride

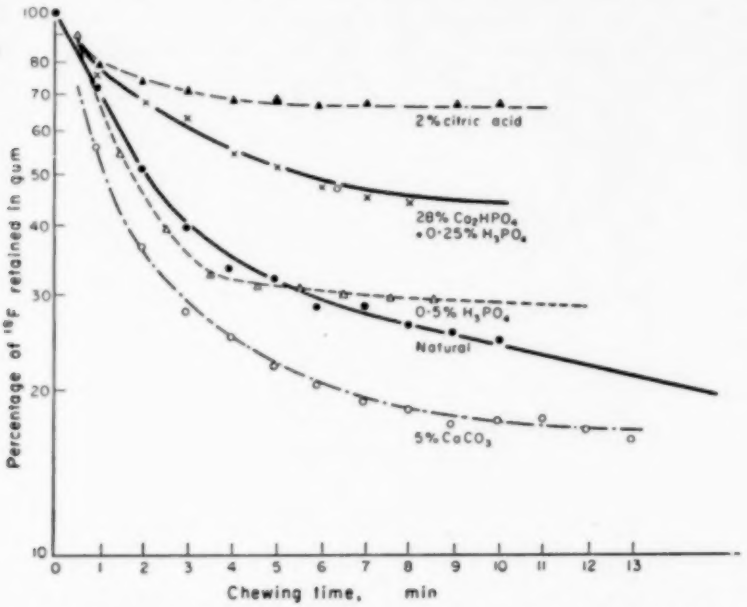


FIG. 2. The effect of various additions upon the rate of release of ^{18}F from chewing gum.

gum, is shown in Table 1. In general ^{18}F counting revealed radioactivity in a premolar tooth, corresponding to an uptake of the order of 20 mμg F from 10 min chewing of gum containing 100 mμg F.

TABLE 1. UPTAKE OF LABELLED FLUORIDE BY PREMOLAR TEETH, AFTER CHEWING STANNOUS FLUORIDE CONTAINING GUM FOR 10 MIN

Name	Age (years)	Tooth available	Uptake per 100 μg F (mμg)
C.S.	13	$\overline{\lvert 4}$	31
B.P.	18	$\overline{\lvert 5}$	19
R.M.	10	$\underline{4\rvert}$	22
		$\underline{\lvert 4}$	22
		$\overline{\lvert 4}$	9
C.D.	11	$\underline{\lvert 4}$	24
		$\underline{4\rvert}$	10

Uptake of ^{18}F *by teeth from toothpaste*

The amount of available fluoride in the toothpaste at the time of use was not known. Therefore the uptake by the teeth has been calculated on the assumption that all the fluoride was available. This assumption serves to give an indication of the order of magnitude of the uptake. The twelve sound premolar teeth studied took up ^{18}F activity corresponding to a fluoride uptake of about 100–200 mμg from 1 g of toothpaste, containing 1 mg F as the stannous salt (Table 2).

TABLE 2. UPTAKE OF LABELLED FLUORIDE BY PREMOLAR TEETH, AFTER BRUSHING WITH STANNOUS FLUORIDE TOOTHPASTE FOR 1 MIN

Name	Age (years)	Tooth available	Uptake per A mg F (mμg)
A.W.	12	$\underline{4}\|$	96
		$\overline{4}\|$	96
		$\|\underline{4}$	106
		$\|\overline{4}$	99
D.W.	15	$\underline{4}\|$	321
		$\|\underline{4}$	168
H.B.	14	$\overline{5}\|$	152
		$\|\overline{5}$	120
J.M.	13	$\|\overline{5}$	38
		$\overline{5}\|$	171
A.S.	11	$\overline{4}\|$	105
		$\|\overline{4}$	196

DISCUSSION

The results show that fluoride is rapidly released from gum on chewing. If gum is to be used for the topical application of fluoride a chewing time of 10 min should be sufficient.

The reduced release of fluoride when the potato starch filler was replaced by dicalcium hydrogen phosphate demonstrated the need to keep the calcium content of the gum to a minimum.

The high uptake of fluoride by the teeth, in spite of low release, from the gum containing 2% citric acid was at first thought to be advantageous. It was hoped to include citric acid in the gum to which increased amounts of fluoride could then be added without increasing systemic absorption. However, gum containing 2% citric acid was obviously sour in taste and its effect on salivary pH was marked.

After chewing for 1 min the pH of expectorated saliva fell to between 5·5 and 6. It seemed probable that this gum could produce sufficient acidity at the tooth surface to cause enamel demineralization. For this reason experiments with gum containing these amount of acid were discontinued. Increased uptake of fluoride could not be demonstrated when the gum was acidulated with 0·5% citric or phosphoric acids.

It is difficult to make a comparison of the uptake of fluoride from chewing gum and toothpaste because the amount of available fluoride at the time of testing is not known. With stannous fluoride this may introduce a significant error. The demonstrated uptake of fluoride from stannous fluoride toothpaste may have to be reduced five-fold according to the age of the paste. Fluoride in the chewing gum, especially as sodium fluoride, is probably more stable. If ageing is ignored, chewing the gum for 10 min, or brushing teeth for 1 min, with a paste containing ten times as much fluoride, causes an uptake of fluoride in premolar teeth which is approximately proportional to the initial fluoride content of the gum or paste.

Any value the demonstrated uptake of fluoride may have in caries prevention will depend upon its method of entry into the enamel. FREMLIN, HARDWICK and MATHIESON (1959) in *in vitro* experiments have shown that there is a large amount of iso-ionic exchange between ^{18}F and stable enamel fluoride. Hetero-ionic exchange with, for example, hydroxyl ions of the apatite molecule, also occurs, but on a smaller scale. Some of our teeth, which had taken up ^{18}F from chewing gum, were shaken with a 0·1% sodium fluoride solution for 10 min, but there was very little loss of activity. This suggests that little iso-ionic exchange between fluoride of chewing gum and enamel can have occurred. The increase in enamel fluoride content with age, in fluoride areas, demonstrated by JACKSON and WEIDMANN (1959); coupled with the report of ISAAC *et al.* (1958), which showed that the increase in fluoride is most marked in the outer layer of the enamel, suggests, that *in vivo*, hetero-ionic exchange can make a marked contribution to enamel fluoride content. It would appear that the use of fluoridated chewing gum and toothpaste could also help to to raise the fluoride content of surface enamel.

REFERENCES

EMSLIE, R. D., VEALL, N. and DUCKWORTH, R. 1961. *Brit. dent. J.* **110**, 121.
FREMLIN, J. H., HARDWICK, J. L. and MATHIESON, J. 1959. *J. dent. belge* **50**, 51.
ISAAC, S., BRUDEVOLD, F., SMITH, F. A. and GARDNER, D. E. 1958. *J. dent. Res.* **37**, 318.
JACKSON, D. and WEIDMANN, S. M. 1959. *Brit. dent. J.* **107**, 303.
KLAPPER, C. E. and VOLKER, J. F. 1954. *J. dent. Res.* **34**, 703.
PEFFLEY, G. E. and MUHLER, J. C. 1960. *J. dent. Res.* **39**, 871.

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 266–274, 1961. Pergamon Press Ltd.

DISSOLUTION OF DENTAL ENAMEL IN DECALCIFYING BUFFERS CONTAINING DIFFERENT AMOUNTS OF CALCIUM AND PHOSPHORUS

T. M. MARTHALER, ANGELA SCHAIT and H. R. MÜHLEMANN

Experimental Caries Research Laboratory, Dental Institute, University of Zürich, Switzerland

Abstract—Crowns of intact premolars and molars were exposed for 3 hr to 10 ml of acid buffer, pH 4, containing differing amounts of calcium and phosphate at the beginning of the experiments. Acetic and phthalic acid buffers were used at 0·025 and 0·075 molar concentrations. Calcium and phosphate ions were added by dissolution of $CaCl_2$ or Na_2HPO_4.

The presence of calcium and phosphate alone was of little effect on the dissolution rate. A more clearly diminished dissolution rate was found when both calcium and phosphate were present in the buffers. The relative protection against dissolution by the presence of calcium and phosphate became considerably smaller when the molarity of the buffers was raised from 0·025 to 0·075. The presence of calcium or phosphorus in the acid buffers did not change the calcium/phosphorus ratio of the dissolved enamel substance.

Addition of NaCl or $MgSO_4$ to acetic acid buffer already containing calcium and phosphate resulted in an increase of the enamel dissolution rate.

The results are compared with certain findings in experimental caries studies.

Zusammenfassung—Intakte Kronen von menschlichen Prämolaren und Molaren wurden während 3 Stunden in 10 ml saure Pufferlösungen mit einem pH von 4 verbracht, die zu Beginn der Versuche verschiedene Mengen von Kalzium und Phosphat enthielten. Essigsäure-und Phthalatpuffer wurden bei 0,025 und 0,075 molarer Konzentration verwendet. Kalzium- und Phosphationen wurden durch Auflösung von $CaCl_2$ oder Na_2HPO_4 in die Pufferlösungen gebracht.

Die Gegenwart von Kalzium oder Phosphat allein war von nur geringer Wirkung auf die Auflösung des Schmelzes. Eine deutlicher verminderte Auflösungsrate zeigte sich, wenn Kalzium und Phosphat zugleich in der Lösung vorhanden waren. Der relative Schutz gegen Auflösung von Schmelz durch die Gegenwart von Kalzium und Phosphat sank bedeutend, wenn die Molarität der Puffer von 0,025 auf 0,075 erhöht wurde. Die Gegenwart von Kalzium oder Phosphat in der Lösung wirkte sich auf den Kalzium/Phosphorquotient der in Lösung gegangenen Schmelzsubstanz nicht aus.

Zugabe von NaCl oder $MgSO_4$ zu Essigsäurepuffer, der schon Kalzium und Phosphat enthielt, bewirkte eine Erhöhung der Schmelzauflösungsrate.

Die Resultate werden mit gewissen Befunden aus der tierexperimentellen Kariesforschung verglichen.

INTRODUCTION

A DISCUSSION of the equilibrium between calcium and phosphate in decalcifying solutions and enamel dissolution has been presented by HARDWICK (1949; 1950). He concluded that the presence of calcium, phosphate, carbonate or other ions will modify or inhibit enamel dissolution. In studying the production of caries-like changes by chemical means, BESIC (1953) found that acid solutions saturated with calcium and phosphate appeared to produce no effect on enamel *in vitro*. A quantitative study of enamel dissolution in the presence of calcium and phosphate ions is given by STRALFORS (1959). He found that the presence of calcium, phosphate or both in lactic acid buffer reduced the weight loss of enamel pieces.

The present experiments were set up to investigate the interaction between calcium and phosphate in solution, as well as the interaction between calcium and phosphate and the buffer concentration.

MATERIAL AND METHODS

Extracted molars and caries-free premolars, stored in physiological saline at 0–2°C not longer than 3 weeks were used. The root surfaces were covered with sticky soft wax after cleaning the teeth. In the molars, the occlusal fissures were covered with sticky wax to obtain some standardization of the enamel surface size since part of the occlusal fissures were filled. The remaining surfaces were also intact in the molar crowns.

Blocks of similar teeth—molars, premolars—were put together in equal numbers as given by the number of treatments in the plans of the experiments. Each tooth of a block was assigned at random to one of the treatments. The experiments were carried out separately for each acid buffer. The teeth were decalcified for 3 hr in 10 ml of buffer solution at pH 4·0 in a horizontal shaker at 37°C.

It was found that with the wax coating procedure used, calcium and phosphorus removal was hardly less in premolars than in molars. The statistical analysis was therefore simplified and carried out irrespective of the blocks. The blocks were, however, always put together for the randomization procedure as a safeguard against possible influences of the tooth size.

Preparation of solutions

The preparation of the solutions was started with concentrated buffers or acids of a higher acidity than chosen for the experiment. To these buffer solutions, the solutions containing the salts under investigation were added. Calcium was present as $CaCl_2$, phosphate as Na_2HPO_4. It was found that by the addition of the salts, the pH was raised by up to 0·3 units. The pH was then adjusted with NaOH to 4·0. The concentration chosen was obtained by addition of distilled water.

The rise in pH may have several causes. When a great number of ions are present in the acid buffer, the mobility, or, as it is more usually called, the activity of the ions is decreased, and the dissociation of the acid will also be lowered to some extent. The dissolved salt may also exert a buffering action of its own. It may be mentioned in this connexion that the electrometric measurement of the pH gives the activity of the hydrogen ions, and not their true concentration, which is somewhat higher. Hence the pH change will mirror the combined effect of all the interferences stated above on the activity of the hydrogen.

As a result of the buffering capacity of the phosphate, the greatest increase of the pH, 0·2 to 0·3, was observed after the addition of Na_2HPO_4, while smaller changes were observed after the addition of the other salts.

The phosphate going into solution during the experiment also caused some rise of the pH. In the buffers of 0·025 molarity, the maximal rise observed was 0·4 units. In the more concentrated buffer systems, the rise did not exceed 0·2 units.

The solutions containing both calcium and phosphate were prepared by adding solutions of $CaCl_2$ and Na_2HPO_4 to the buffer. It was not possible to dissolve directly an equivalent amount of $Ca_3(PO_4)_2$ in the acids. The effect of the simultaneous presence of sodium and chloride ions in the solution will also be investigated.

After the preparation of the solutions, they were analysed for calcium and phosphorus. Satisfactory agreement between the calculated amount of calcium and phosphorus and the results of the analyses was found.

Analyses

Analyses of phosphorus were carried out according to the method of FISKE and SUBBAROW (1925) modified by LOHMANN and JENDRASSIK (1926). Calcium determinations were made after the complexometric method of SCHWARZENBACH (1960). The determination of phosphorus has the advantage of being based on a measurable absorption, while for calcium an endpoint of a colour reaction has to be determined by eye.

Influence of ions other than calcium and phosphate

As calcium and phosphate were introduced into the decalcifying buffers by addition of solutions of $CaCl_2$ and Na_2HPO_4, it was necessary to investigate the influence of the sodium and chloride ions on the dissolution. This was done by further addition of sodium chloride to the buffer solutions already containing $CaCl_2$ and Na_2HPO_4.

Moreover, the mere addition of any ions may bring about different conditions in the decalcifying buffer. In order to investigate this factor, magnesium was added as such a supplementary ion because of its similarity to calcium. It was dissolved into the buffer as magnesium sulphate.

The concentrations used and the results are assembled in Table I. Only the phosphorus dissolved from dental enamel was determined.

TABLE 1. PHOSPHORUS REMOVAL FROM INTACT DENTAL ENAMEL AFTER ADDITION OF SODIUM CHLORIDE AND MAGNESIUM SULPHATE TO DECALCIFYING BUFFER SOLUTION; ACETIC ACID BUFFER 0·025 M, pH 4, 3 hr at 37°C

Ions added to acid buffer (m equiv/l)						Increase of phosphorus concentration*†	Amount of phosphorus removed from teeth (μg)	
Ca	P	Na*	Cl	Mg	SO_4		Mean	S.E.
0	0	0	0	0	0	5·0	524	24
10·0	10·0	6·6	10·0	0	0	1·3	139	11
10·0	10·0	13·0	16·4	0	0	1·8	192	23
10·0	10·0	19·4	22·8	0	0	2·4	253	39
10·0	10·0	25·8	29·2	0	0	2·3	236	14
0	0	0	0	9·5	9·5	5·3	552	17
10·0	10·0	6·6	10·0	9·5	9·5	2·9	297	25

* Sodium introduced as NaOH for adjustment of the pH not listed.
† Mean of six teeth, each in 10 ml buffer solution.

It may be seen that the enamel solution rate is decreased in the presence of calcium and phosphate ions. This decrease is partially diminished in the presence of supplementary sodium and chloride or magnesium and sulphate ions. The mere addition of magnesium sulphate to the buffer did not result in a significant increase of the enamel dissolution rate.

It may be concluded from the figures in Table I that only the presence of calcium or phosphate in the solution is responsible for the decrease in enamel solution rate. The dissolving power of the buffer solution is unchanged or slightly increased by the presence of the other ions at the concentration used.

Experiments with calcium and phosphate at two different concentration levels in solution

The two concentrations of calcium and phosphate in the acid buffers at the beginning of the experiments were zero and 10 m equiv/l. The acid buffers were used at two different concentration levels, 0·25 and 0·075 M. As both concentration levels of the acid buffer were tested in one experiment, the total number of treatments in the factorial arrangement was thus $2 \times 2 \times 2 = 8$. The experiments were carried out separately with acetic, phthalic and lactic acid buffers. Four blocks of eight teeth were used in each experiment, the number of teeth per treatment being four.

The results from the experiments with acetate and phthalate buffers are presented in Fig. 1. For reasons not yet known, no consistent results were obtained with lactate buffer. The results from the experiments with this buffer are not pre-

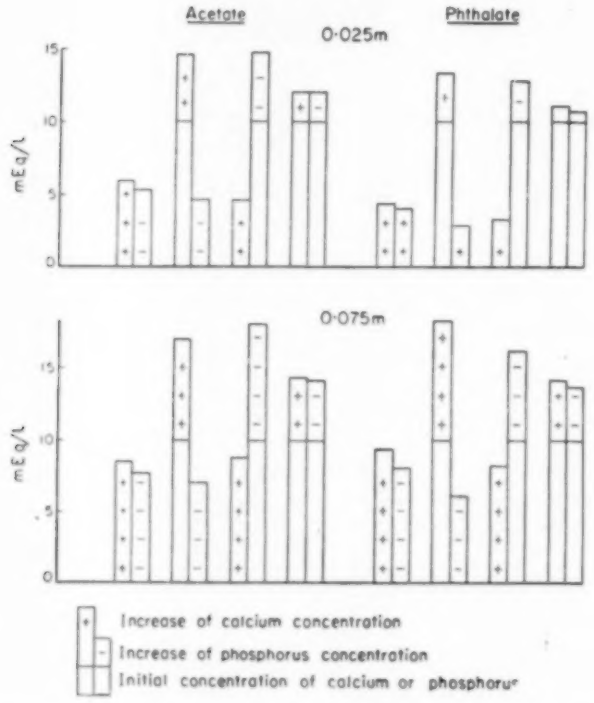


FIG. 1. Increase in calcium and phosphorus concentration in acetate and phtalate buffers (molarities 0·025 and 0·075) pH 4, from dissolution of intact dental enamel for 3 hr at 37°C. One tooth immersed in 10 ml buffer solution. Each mean is based on the results of four teeth.

sented. Analyses of variance of the results with acetate and phthalate buffer are given in Table 2. After elimination of the treatment effects, the remaining variance within treatments was taken as experimental error.

In the case of the acetate buffer, the main effects of initially added calcium and phosphate and their interaction are significant. Less calcium and phosphate was dissolved from the enamel when the decalcifying buffer contained 10 m equiv/l calcium or phosphate initially than the initial calcium and phosphate concentration was zero. The interraction has the same sign as the main effects, that means that the effect of calcium in the absence of phosphate is less than the effect of calcium in the presence of phosphate. The same is true for phosphate in the absence or

TABLE 2. ANALYSIS OF VARIANCE OF CALCIUM AND PHOSPHORUS REMOVED FROM INTRACT DENTAL ENAMEL (IN TENTHS OF A MILLIGRAM) IN ACETATE AND PHTALATE BUFFERS CONTAINING DIFFERENT CALCIUM AND PHOSPHATE CONCENTRATIONS AT THE BEGINNING OF THE EXPERIMENTS. THE RESULTS ARE GRAPHED IN FIG. 1

Source of variation	Degrees of freedom	Mean squares			
		Acetate buffer		Phthalate buffer	
		Calcium	Phosphorus	Calcium	Phosphorus
Calcium in buffer solution	1	174·84	32·66	140·70	32·20
Phosphate in buffer solution	1	76·26	20·79	150·94	30·34
Interaction Ca × P	1	49·00	13·61	34·24	0·82
Concentration of acid	1	584·51	59·00	631·01	94·33
Concentration × caclcium	1	14·04	1·37	7·13	0·81
Concentration × phosphate	1	1·90	0·46	10·47	0·48
Concentration × Ca × P	1	3·12	1·62	4·73	0·11
Error, within treatments	24	3·45	0·962	1·88	0·217
Mean square with probability of $P = 0{\cdot}05$ ($s^2F_{0{\cdot}05}$; D.F. = 1 and 24)		14·69	4·10	8·01	0·99

presence of calcium. The interaction with buffer concentrations are not significant. The lower amount of calcium and phosphate removed in buffers with calcium and phosphate present initially is thus equal at both concentrations.

Different conditions are met with in the phthalate buffer. The effects of calcium and phosphate initially present in the buffer appear to be independent of each other when the phosphorus removal is considered. In the analysis of variance of the calcium removal, a significant interaction between calcium and phosphate, and buffer concentration is found. These interactions are, however, unimportant when compared with the main effects of calcium and phosphate.

It may be concluded that the dissolution of enamel is somewhat slower when calcium or phosphate is present in the solution. When both calcium and phosphate are present, the protective effect is at least as great as the sum of the single effects or may be significantly greater than the sum of the single effects as in the case of the acetic acid buffer. The effects of calcium and phosphate being constant over the buffer concentrations, the relative effects of calcium and phosphate become unimportant when the buffer concentration rises.

Calcium/phosphorus ratio

When enamel is dissolved, both cationic and anionic mineral constituents must go into solution. These ions are mainly calcium and phosphate, other ions being present in only small quantities in the enamel. Therefore spectacular changes in the calcium/phosphorus ratio of the material dissolved from the enamel are not to be expected. Some authors have described a preferential dissolution of carbonate from

the enamel under certain conditions. A preferential dissolution of carbonate would be favoured when phosphate is already present in the solution. As a consequence, the calcium/phosphorus ratio would increase.

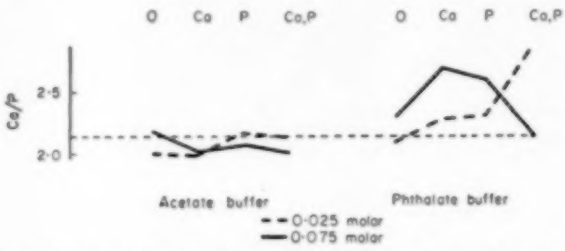


FIG. 2. Ratios of the weight of calcium and phosphorus dissolved from intact dental enamel (experiments with calcium and phosphate at two levels). The broken line represents the theoretical ratio for apatite.

The quotients of the weight of calcium and phosphorus dissolved from the crowns are shown in Fig. 2. The broken line represents the theoretical quotient for apatite. Very regular values are found in the acetic acid buffer. No evidence of a change of the calcium/phosphorus ratio is found in these results. A less regular pattern is found in the case of the phthalic acid. But also in this series, the ratio does not appear to be influenced by the excess of calcium or phosphate in the buffer solutions.

Experiments with calcium and phosphate at three levels

The experiments were carried out with acetic and phthalic buffers. The levels of calcium and phosphate chosen were zero, 5·5 and 9·7 m equiv/l. The treatment number with two factors at three levels is $3^2 = 9$. Owing to the great

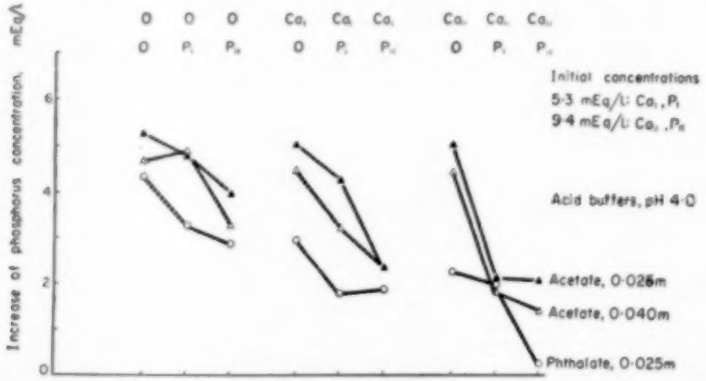


FIG. 3. Increase in phosphorus concentration in acetate and phthalate buffer, pH 4, from dissolution of intact dental enamel for 3 hr at 37°C. One tooth immersed in 10 ml buffer solution. Each mean is based on the results of six teeth.

number of treatments, only one buffer concentration could be included in one experiment. Six blocks of nine teeth each were used for each experiment. Only phosphorus determinations were carried out.

The results are presented in Fig. 3. In the case of acetic acid buffer, the single effects of calcium and phosphate alone are hardly detectable while the presence of both calcium and phosphate caused a marked decrease of the phosphorus removal. In the phthalic acid buffer, calcium and phosphate were equally effective alone or in presence of the other ions. These findings are in agreement with the findings presented in the preceding section.

DISCUSSION

It was suggested by STRALFORS (1959) to add calcium and phosphate to fermentable carbohydrates. Incorporated into the plaque, calcium and phosphate would go into solution when the pH falls, and the local increase of calcium and phosphate is expected to bring about a chemically induced inhibitory action on caries activity. Considering the present experiment, it might be assumed that the addition of calcium or phosphate alone in equivalent amounts should result in a limited but more or less equal inhibitory effect on caries. A considerably stronger effect would be expected after addition of both calcium and phosphate at the chosen level.

Concluding from the experiments with sodium and chloride ions in solution, a diminished anticariogenic effect of the calcium phosphate is to be expected in the presence of sodium and chloride ions. MCCLURE (1958) and MCCLURE and MULLER (1959a, b) presented findings contradictory to this hypothesis. Dibasic calcium phosphate was found to be cariostatic in rats only when the diet was supplemented with sodium chloride. These experiments show that the conditions in the plaque are very different from our experimental conditions. In the plaque, the pH is not stable. By their buffering capacity phosphates may already prevent a fall of the pH to a certain extent (KÖNIG, MARTHALER and MÜHLEMANN, 1961). An interference of some phosphates with the bacterial metabolism is suggested by the experiments of LILIENTHAL (1956) as well as KÖNIG *et al.* (1961) who also investigated chemical modifications of the superficial enamel layers. The relative importance of all these mechanisms is not known. Further information will be obtained when factorial experiments of the same design as presented in these chemical studies are carried out.

REFERENCES

BESIC, F. C. 1953. Carieslike enamel changes by chemical means *J. dent. Res.* **22**, 830–839.

FISKE, C. H. and SUBBAROW, Y. 1925. The colorimetric determination of phosphorus. *J. biol. Chem.* **66**, 375–400.

HARDWICK J. L. 1949, 1950. Enamel caries: a chemico-physical hypothesis. Phase one—chemical. *Brit. dent. J.* **87**, 137–142; **89**, 1–6.

KÖNIG, K. G., MARTHALER, T. M. and MÜHLEMANN, H. R. 1961. Effects of some phosphates in a short-period rat caries experiment. *Arch. oral Biol.* **3**, 258–270.

LILIENTHAL, B. 1956. The effect of fluoride on acid formation by salivary sediment. *J. dent. Res.* **35**, 197-204.

LOHMANN, K. and JENDRASSIK, L. 1926. Colorimetrische Phosphorsäurebestimmungen im Muskelextrakt. *Biochem. Z.* **178**, 419–426.

MCCLURE, F. J. 1958. Wheat cereal diets, rat caries, lysine and minerals. *J. Nutr.* **65**, 619–631.

MCCLURE, F. J. and MULLER, A. 1959a. The caries-inhibiting effect of dibasic sodium phosphate and dibasic calcium phosphate added to wheat flour and bread diets. *J. Amer. dent. Ass.* **58**, 36–41.

MCCLURE, F. J. and MULLER, A. 1959b. Further observations on the cariostatic effect of phosphates. *J. dent. Res.* **38**, 776–781.

SCHWARZENBACH, G. 1960. *Die Komplexometrische Titration.* Ferdinand Enke, Stuttgart.

STRALFORS, A. 1959. The effect of calcium and phosphate ions on the rate of enamel dissolution. *Odont. Revy* **10**, 367–378.

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 275–283, 1961. Pergamon Press Ltd.

GAMMA-RAY SPECTROMETRIC ANALYSIS OF ELEMENTS IN NORMAL HUMAN ENAMEL

R. SÖREMARK and K. SAMSAHL

The Clinical Laboratory and the Department of Prothetics of the Royal School of Dentistry and AB Atomenergy, Stockholm, Sweden

Vol. 6
1961

Abstract—The concentrations on the basis of dry weight of Ca, P, Cl, Na, Sr, Zn, Br, Mn, W, Cu and Au in normal human enamel have been determined. Quantitative data of the concentrations ± standard deviation have been tabulated. The values have been obtained from fifteen intact first bicuspids from 14- to 16-year-old boys and girls.

The concentrations have been studied by means of neutron irradiation of the enamel samples for about 20 hr in a neutron flux of 2×10^{12} neutrons/cm²/sec. After chemical group separation of the activated enamel gamma-ray spectrometric analysis followed.

Zusammenfassung—Es wurde die Konzentration von Ca, P, Cl, Na, Sr, Zn, Br, Mn, W, Cu und Au in normalen Schmelz bestimmt. Die quantitativen Werte und ihre Standardabweichungen an 15 gesunden Zähnen von 14–16 J. alten Mädchen und Knaben wurden festgestellt.

Die Konzentrationen wurden mit Hilfe der Neutronenbestrahlung der Zahnschmelzproben, die mit bekannten Substanzen verglichen wurden, untersucht. Der Neutronenfluß wurde 2×10^{12} Neutronen pro cm²/sec gehalten.

Es wurde eine Methode zur Gruppentrennung aller entstandenen radioaktiven Elemente ausgearbeitet, die der Gammastrahlenanalyse vorgeschaltet wird. Das Trennungssystem beruht auf dem Ionenaustauschprinzip und erlaubt eine sehr schnelle Analyse. Zur Messung der Radioaktivität wurde ein 256-Kanal Gammastrahlenspektrometer benutzt.

INTRODUCTION

THE HIGH proportion of mineral constituents of teeth can be divided into two categories depending on their concentration, namely the macroelements and the trace elements. The macroelements in hard tissues of teeth, i.e. calcium and phosphorus, have been studied for a number of years and their concentrations have been well established. The second group, on the other hand, the trace elements, most of which occur in a few p.p.m. or less, have so far not been extensively studied. This invalidity of quantitative data regarding trace elements may probably be explained by the lack of reliable analytical methods.

Quantitative determinations on caries-free human enamel of calcium, phosphorus, and magnesium have been reported e.g. by BURNETT and ZENEWITZ (1958a, b). BRUDEVOLD and STEADMAN (1956a, b) determined the concentrations of lead, copper and tin in human enamel. STEADMAN *et al.* (1959) gave quantitative data for some trace elements in ancient Indian teeth. NIXON and SMITH (1960) used activation analysis in estimation of arsenic in human teeth. JENKINS (1960) has given an excellent review of the trace elements in enamel and dentine.

In view of the importance of various elements in the formation and preservation of teeth, an analytical study based on neutron activation and gamma-ray spectrometric analysis of the inorganic composition of human teeth has been initiated and is in progress.

The choice of a particular technique for the analysis of trace elements depends not only on its sensitivity as well as its reproducibility but also on its range and convenience.

By means of radioactivation analysis many elements in minute concentrations can be detected and determined with greater sensitivity and precision than by other analytical methods (KOCH, 1960). Neutron activation analysis is both sensitive and reproducible. The limitation of activation analysis depends, among other factors, to a high degree on the neutron flux used for bombardment of the samples. In the present investigation a thermal neutron flux of about 2×10^{12} neutrons /cm²/ sec in the atomic reactor R1 of the Swedish Atomic EnergyCommiss ion has been used. During the last 3 months a flux of about 10^{14} neutrons /cm²/ sec. has been available for our studies, and the results from the experiments using this more satisfactory neutron flux will be published later. Even with the lower flux used in the present study a sensitivity will be reached which is greater than for any other known analytical method concerning a variety of elements.

Activation analysis of biological materials causes some difficulties in the chemical separation procedures following the neutron bombardment of the sample. These difficulties are most pronounced for hard tissues such as enamel and dentine because of the high concentration of calcium and phosphorus. A method for group separation of chemical elements as a pretreatment for gamma-ray spectrometric analysis has been proposed by SAMSAHL (1961). Gamma-ray spectrometric analysis is the most common way of measuring the activity from elements made radioactive by neutron bombardment (CROUTHAMEL, 1960). A 256-channel gamma spectrometer has been used in the present investigation.

In order to obtain quantitative data on the elements identified, the sample is irradiated together with a known quantity of the element to be determined, and a direct comparisons is then made. These methods are described and discussed also in our paper concerning dental calculus (SÖREMARK and SAMSAHL, 1961a).

Vol. 6
1961

EXPERIMENTAL PROCEDURE

Fifteen intact first bicuspids from humans between 14 and 16 years old were used in the present investigation. Seven girls and eight boys were selected on characteristics of good general health. They engaged in normal duties and enjoyed a normally varied diet. All the children were born and lived in the city of Stockholm. The teeth were extracted for orthodontic reasons and were prepared for activation within 48 hr after extraction. The subjects had no other restorations than amalgam and silicate. However, the teeth to be analysed had no restorations and no fillings were present in the neighbouring teeth.

Nearly all of the enamel was separated from the dentine mechanically by chipping and breaking the teeth with glass or polyethylene covered instruments. The samples were collected in polyethylene bags and sealed. The enamel samples were not in contact with materials other than polyethylene. The samples were dried in an electric oven for 48 hr at 105°C. Standard amounts of the elements to be studied were inserted into polyethylene tubes and sealed and then placed in the same standard aluminium can as the sample. The standards were consequently irradiated simultaneously with the sample. The aluminium can containing the standards and the unknown sample was then sent to the neutron pile R1 in Stockholm for irradiation with thermal neutrons for about 20 hr. The flux was about 2×10^{12} neutrons /cm^2/sec.

When the period of irradiation was completed, the samples were dissolved in 1N HCl containing 0·3% H_2O_2. The solution thus obtained was then subjected to chemical group separation. The various irradiated standards were collected in a bottle and also a small piece of carrier enamel was dissolved and added. This standard solution was treated in exactly the same way in the group separation system as the unknown samples. The following scheme was used for the identification and determination of different elements in the eight groups of the separation series:

Group 1. NaOH absorption: Cl, Br, I.

Group 2. Dowex 2-chloride: Mo, Tc, Ru, Rh, Ag, Cd, W, Re, Os, Ir, Au, Hg.

Group 3. Dowex 50-hydrogen: Fe, 55–65% Cu, Zn, Ga, In, 20–30% Te.

Group 4, Dowex 50-hydrogen effluent: 15–25% As, 10–20% Se, Sb, 70–80% Te.

Group 5, Dowex 2-citrate: 3–7% Mg, Sc, Co, Ni, 35–45% Cu, 75–85% As, 80–90% Se, Zr, Nb, Wp, La, the rare earths, Hf, 95–100% Ta, 80–90% Np.

Group 6, Dowex 2-hydroxide: Cr, Mn, 10–20% Np.

Group 7, Dowex 50-sodium: Na, K, Rb, Cs.

Group 8, Dowex 2-Dowex 50, mixed bed: Ca, Sr, Ba.

Dowex $2 \times 10{,}200$–400 mesh, and Dowex $50 \times 12{,}200$ — 400 mesh, were used.

The ion exchange columns were arranged as shown in Fig. 1. Detailed descriptions about the group separation procedures are given by SAMSAHL (1961).

After the radioactive elements had been collected in the various ion exchange columns the ion exchange resins were dried at 200°C, homogenized, and transferred to stoppered polyethylene tubes for counting in a 3 × 3 in. well-type NaI-scintillation

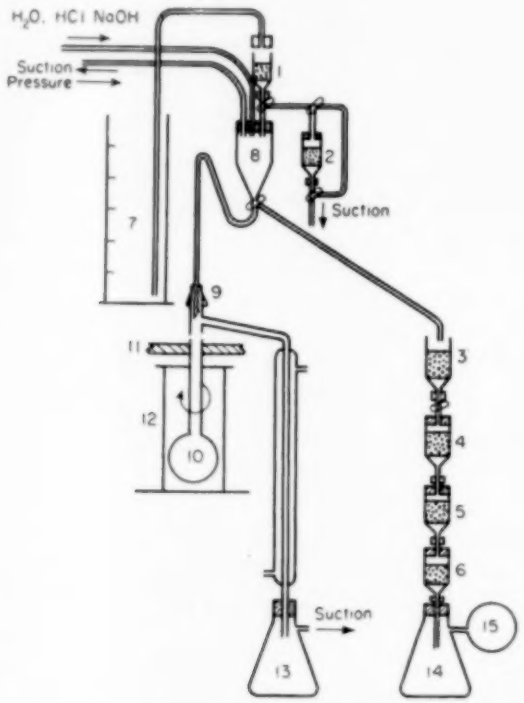


FIG. 1. Schematic drawing of the arrangements of the ion exchange system for group separation of the neutron activated samples and standards.

1. Dowex 2, chloride column.
2. Dowex 50, hydrogen column.
3. Dowex 2, citrate column.
4. Dowex 2, hydroxide column.
5. Dowex 50 sodium column.
6. Dowex 2 + Dowex 50, mixed bed column.
7. Reservoir, 500 ml.
8. Reservoir, 50 ml.
9. Silicon rubber connexion.
10. Flask, 50 ml, long neck, round bottom.
11. Pulley wheel.
12. Pyrex glass tube, 6 cm in diam., 14 cm in height and covered with asbestos plates at the ends.
13. Suction flask, 100 ml.
14. Suction flask, 100 ml.
15. Rubber ball for suction.

crystal. The activity was measured and corrected for natural background with a transistorized 256-channel gamma spectrometer. Quantitative data on the basis of the dry weight of the samples were obtained by comparing the gamma intensity of the identified elements with those of the appropriate standards.

Vol. 6
1961

RESULTS

The results obtained by means of neutron activation and gamma-ray spectrometric analysis of the enamel of fifteen caries-free first bicuspids from 14–16-year-old boys and girls on the basis of dry weight are tabulated in Table 1. With irradiations at a neutron flux of 2×10^{12} neutrons /cm²/sec for 20 hr the following elements were determined in the enamel: Ca, P, Cl, Na, Br, Sr, Mn, Cu, W, Au and Zn.

TABLE 1. THE CONCENTRATION OF Ca, P, Cl, Na, Sr, Zn, Br, Mn, W, Cu AND Au IN NORMAL ENAMEL OF FIFTEEN FIRST BICUSPIDS FROM 14–16-YEAR-OLD HUMANS

The values are based on the dry weight and the enamel samples were neutron activated for 20 hr in a flux of 2×10^{12} neutrons /cm²/sec and analysed in a 256-channel gamma spectrometer.

The mean values from fifteen first bicuspids ± the standard deviations are tabulated.

Ca (%)	P (%)	Na (%)	Cl (%)
37·4 ± 1·0	18·3 ± 2·2	1·16 ± 0·4	0·65 ± 0·3

Zn (p.p.m.)	Sr (p.p.m.)	Br (p.p.m.)	Mn (p.p.m.)	Cu (p.p.m.)	W (p.p.m.)	Au (p.p.m.)
276 ± 106	93·5 ± 21·9	4·6 ± 1·1	0·54 ± 0·08	0·26 ± 0·11	0·24 ± 0·12	0·02 ± 0·01

No significant difference with respect to sex was found. Neither were any significant differences found between the teeth from upper and lower jaw.

Records of four groups of an enamel sample are exemplified in Fig. 2.

Enamel from four second molars and three canines were also analysed. The concentrations of the various elements in these teeth did not significantly deviate from those tabulated for the first bicuspids.

DISCUSSION

BURNETT and ZENEWITZ (1958a, b) have discussed the results obtained in relation to the various procedures to prepare the samples. In the present investigation separation of enamel and dentine by chipping and breaking was used. This procedure has seldom been used after the development of the flotation method. The flotation method is, however, not satisfactory for preparing samples for activation analysis because of the great risk of contamination. The method used in the present study seemed to be accurate for activation analysis when using instruments not containing inorganic components. With this method an amount of enamel sufficient for activation and gamma-ray spectrometric analysis was obtained from each tooth. However, using relatively large pieces of enamel leads to some difficulty in establishing equal samples with regard to moisture, because the drying procedure allows some moisture to remain in the sample pieces. The varying moisture content could influence the de-

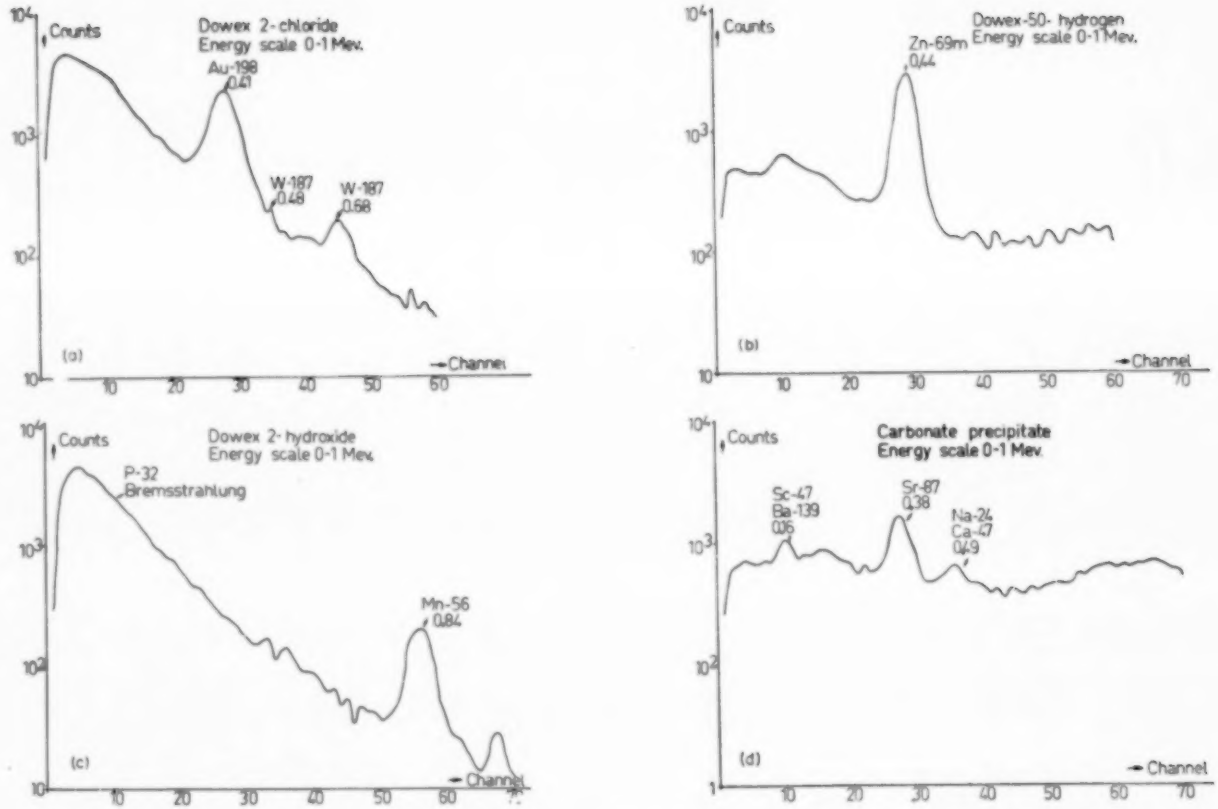


FIG. 2. Gamma-ray spectra of four groups of a neutron activated enamel sample which was taken from an intact first bicuspid (+4) from a 15-year-old boy.

termination of the various elements when the dry weight is used as the basis of calculation. The moisture content of freshly extracted caries-free whole teeth, minus pulp, was found to be 9·3 per cent and for dentine 12 per cent (BURNETT and ZENEWITZ, 1958a, b). In the present investigation nearly all the enamel from each tooth was taken for neutron activation in order to avoid errors due to an uneven distribution of the various elements. However, only about 10–20 mg of the samples are needed to obtain reliable data using the present methods.

The concentrations of some trace elements have been found in pooled samples to be higher in outer portions of enamel than in the inner layers (e.g., BRUDEVOLD and LITTLE, 1951; STEADMAN *et al.*, 1959). This has also been found in enamel from rat teeth (MANSELL and HENDERSHOT, 1960). Marked differences occur in the concentrations of fluor, zinc and lead with regard to the surface and subsurface enamel. To a lesser extent Fe, Ag, Mn, Si and Sn are present in greater concentrations in the outer than in the inner portions of the enamel. However, carbonate, Na and Mg follow an opposite distribution pattern and increase in concentrations from the enamel surface inward and Cu and Sr occur in similar concentrations in surface and subsurface enamel (BRUDEVOLD, 1960). BRUDEVOLD and STEADMAN (1956a, b) found in human normal enamel that the amount of lead in the outer enamel of erupted teeth was greater than that of unerupted teeth and showed an increase with age. In the present investigation the whole enamel of the teeth has been analysed without differentiating the enamel in successive layers. It will be noted that in the present investigation the samples were not pooled; grinding layers from enamel by means of instruments will probably contaminate the samples.

It seems probable that the concentrations of various elements may vary with age and geographical regions. In the present investigation all the humans were born and living in the city of Stockholm. In man the testes, hair and bone carry higher concentrations of zinc than other body tissues and organs. Measured in p.p.m. of fresh tissue the values are about 300 for the testes and 100 for bone. Whole blood contains about 10 p.p.m. Great variations have, however, been reported (UNDERWOOD, 1956). In the present study a mean concentration was found of about 300 p.p.m. zinc in human enamel, and it would therefore seem that enamel has a very high concentration of zinc compared with that of other body tissues.

Surprisingly small deviations were found in the concentration of manganese in enamel with respect to the great variations of the daily intake of manganese reported (UNDERWOOD, 1956).

The distribution of halogens have been discussed by SÖREMARK (1960). Also the distribution of sodium has previously been discussed; the teeth are the organ of the body containing the highest concentration of sodium (HUGGERT *et al.*, 1961).

Quantitative data of the same elements as in the present study have been obtained for dentine by means of the same analytical methods (SÖREMARK and SAMSAHL,

1961b). It will be noted that the concentrations of most of the elements studied are higher in enamel than in dentine as can be seen in Table 2.

TABLE 2. THE RATIOS OF THE CONCENTRATIONS OF THE VARIOUS ELEMENTS IN DENTINE AND ENAMEL

Element	Dentine/Enamel
Ca	0·75
P	0·74
Na	0·65
Cl	0·60
Zn	0·68
Sr	0·75
Br	0·87
Mn	0·35
Cu	0·81
W	10·80
Au	1·40

As mentioned in the introduction, our determinations by means of neutron activation and gamma-ray spectrometric analysis of the inorganic composition of enamel as well as of dentine are in progress, and quantitative data of some other elements obtained by a higher neutron flux than that used in the present investigation will be published elsewhere.

REFERENCES

BRUDEVOLD, F. 1960. Chemistry of the enamel surface. *J. dent. Res.* **39**, 1083.

BRUDEVOLD, F. and LITTLE, M.F. 1951. Chemical studies of the human enamel surface. *J. dent. Res.* **30**, 477.

BRUDEVOLD, F. and STEADMAN, L.T. 1956a. The distribution of lead in human enamel. *J. dent. Res.* **35**, 430.

BRUDEVOLD, F. and STEADMAN, L.T. 1956b. A study of tin in enamel. *J. dent. Res.* **35**, 749.

BURNETT G. W. and ZENEWITZ, J. A. 1958a. Studies of the composition of teeth—VIII. The composition of human teeth. *J. dent. Res.* **37**, 590.

BURNETT, G. W. and ZENEWITZ, J. A. 1958b. Studies of the composition of teeth—VII. The moisture content of calcified tooth tissues. *J. dent. Res.* **37**, 581.

CROUTHAMEL, C. E. 1960. *Applied Gamma-ray Spectrometry*. Pergamon Press, London.

HUGGERT, A., ODEBLAD, E., SÖREMARK, R. and ULLBERG, S. 1961. Distribution and kinetics of sodium (Na^{22}) in mice and rabbits. *Acta Isotopica*, **2**, 151.

JENKINS, G. N. 1960. *The Physiology of The Mouth*. Blackwell, Oxford.

KOCH, R. S. 1960. *Activation Analysis Handbook*. Academic Press, London.

MANSELL, R. E. and HENDERSHOT, L. C. 1960. The spectrochemical analysis of metals in rat molar enamel, femurs, and incisors. *Arch. oral. Biol.* **2**, 31.

NIXON, G. S. and SMITH, H. 1960. Estimation of arsenic in teeth by activation analysis. *J. dent. Res.* **39**, 514.

SAMSAHL, K. 1961. A chemical eight group separation method for routine use in gamma spectro-

metric analysis. -AE-54 (1961), AE-56 (1961). (Available from AB Atomenergi, Studsvik, Sweden).

STEADMAN, L. T., BRUDEVOLD, F., SMITH, F. A., GARDNER, D. E. and LITTLE, H. F. 1959. Trace elements in ancient Indian teeth. *J. dent. Res.* **38**, 285.

SÖREMARK, R. 1960. Distribution and kinetics of bromide ions in the mammalian body. *Acta radiol. scand.* Suppl 190.

SÖREMARK, R. and SAMSAHL, K. 1961a. Analysis of inorganic constituents in dental calculus by means of neutron activation and gamma-ray spectrometry. *J. dent. Res.* (In press).

SÖREMARK, R. and SAMSAHL, K. 1961b. Gamma-ray spectrometric analysis of elements in normal-human dentine. *J. dent. Res.* (In press).

UNDEROOD, E. J. 1956. *Trace Elements in Human and Animal Nutrition.* Academic Press, New York.

Vol. 6
1961

Arh. Oral Biol., Special Supplement, Vol. 6, pp. 284–303, 1961. Pergamon Press Ltd.

MEMBRANE PHENOMENA IN CARIOUS DISSOLUTION OF THE TEETH

F. VON BARTHELD

Department of Pathology Dental School, Utrecht, the Netherlands

Vol. 6
1961

Abstract—Experiments are described that have been conducted to ascertain the role of DONNAN membrane effects in carious dissolution of the mineral components of the tooth structures. When a solution of an ionized macromolecular substance is separated from another solution by a semipermeable membrane, which arrests the macromolecules but allows free passage to small ions from both sides, the latter are distributed over both solutions in a peculiar way. Under certain circumstances, depending on the net particle charge, a drop of the pH on one side of the membrane can be effected, accompanied by a rise of the pH on the other side. We applied this principle to the problem of carious tooth decalcification. It was possible to predict and obtain results *in vitro* that closely resemble actual caries, much more so than could be achieved until now with the use of buffered or unbuffered acids. Further experiments, partly still under way, strongly suggest that the beneficial effect of saliva on the tooth substance, mostly described as a mineralizing or remineralizing activity, may also be ascribed to a DONNAN membrane effect, the macromolecular agent being salivary mucin.

Zusammenfassung—Der Haupteinwand, der gegen MILLERS chemoparasitäre Theorie erhoben werden kann, ist die Tatsache, daß es sich als unmöglich erwiesen hat, die typischen kariösen Läsionen *in vitro* durch direkte Säureentkalkung nachzuahmen.

Diese Tatsache hat zur Suche nach biochemischen Theorien zur Erklärung des Mechanismus der kariösen Entkalkung geführt. Diese Theorien waren jedoch genau an demselben Punkt nicht erfolgreich; sie gewannen über ihre theoretischen Aspekte hinaus keine weitere Bedeutung, weil sich sogar noch weniger experimentelle Beweise zu ihrer Stützung erbringen ließen. Der Autor kam zu der Schlußfolgerung, daß es nicht richtig ist, die Anwesenheit von freier Säure in den bakteriellen Plaques anzunehmen; sobald Säure gebildet wird, reagiert sie mit den Bestandteilen der Plaques, und es entstehen Verbindungen, die eine Entkalkung des Zahnschmelzes mittels des Membranphänomens, wie von DONNAN beschrieben, bewirken können. *In vitro* Experimente konnten bei Berücksichtigung aller in Frage kommenden Faktoren den Beweis einer Entkalkung von Zahnschmelz und Dentin erbringen, die morphologisch von der natürlich vorkommenden Karies nicht zu unterscheiden war. Diese Ergebnisse scheinen endgültig bewiesen zu haben, daß die Theorie von MILLER im wesentlichen korrekt ist.

INTRODUCTION

IN THE literature on dental caries indirect evidence of the role of fermentation acids as aetiologic agents is abundant. On the other hand direct proof of their action is entirely lacking, as all *in vitro* experiments, using acids as decalcifying agents, result in etching and cavitation of the tooth surface in a way that is basically different from the clinical aspect and course of dental caries. This study was undertaken to investigate the possible role of membrane phenomena of the DONNAN type, resulting in an acid reaction within the tooth substance and thus causing a decalcification that might resemble dental caries more closely.

According to the views of MILLER a number of organic acids are formed by micro-organisms that constitute and infest the bacterial plaque and the adhering food rests at the well-known sites of carious attack. These acids are the product of carbohydrate fermentation and when present in sufficient concentration may attack the enamel surface, causing the chalky white appearance of the area involved and all other histologic changes that are too familiar to be enumerated here *in extenso*. The main characteristic of these changes is the loss of calcium salts. When the carious attack, *in casu* the opaque area of the enamel, reaches the dentino-enamel junction, the dentine in turn is subject to decalcification which extends in all directions more or less equally, thereby undermining the overlying sound enamel. The dentinal tubules are in most cases found to be infected with micro-organisms, and it is commonly believed that these are responsible for the decalcification of the dentine, as they too are supposed to produce acids from fermentable carbohydrates that allegedly reach them from the oral cavity.

All clinical evidence obtained by MILLER and numerous other workers point to the correctness of these views, insofar as the forming and accumulation of acids is undoubtedly a causative factor in the initiation and the progress of dental caries. At the same time the exact nature of the decalcification mechanism is a much disputed issue, mostly because of the following facts:

1. Time and again it has proved impossible to imitate the carious lesion *in vitro* by the action of acids on sound enamel. Whether weak or strong, organic or mineral, diluted or concentrated, buffered or unbuffered, acids do not cause the same changes as are seen in dental caries. In cases of initial caries the enamel shows a partial dissolution of the apatite underneath a superficial layer of more or less intact enamel, while the histologic structure of the opaque area remains intact to a certain extent (Fig. 1). In experiments with acids however it is commonly found that the hard superficial layer of enamel dissolves first, followed by a progressive softening and total destruction of the enamel, the remains of which are easily washed away by a jet of water (Fig. 2).

2. Even in more advanced cases of carious decalcification, the so called white spot may still show an intact enamel surface. Nevertheless a longitudinal section through this area often discloses extensive decalcification and discoloration of the

underlying dentine with the formation of a dead tract and infection of the tubules (Fig. 3). In these cases, the carious enamel, though opaque and crumbly to the explorer, has retained its anatomical outline, its surface gloss and a considerable

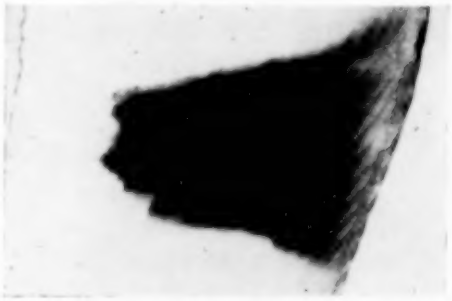


FIG. 1. Ground section of human premolar. Initial enamel caries. Transmitted light. × 60.

density in regard to its permeability. To many workers it has therefore been difficult to accept the idea of soluble carbohydrate passing first the bacterial plaque and then diffusing through the opaque enamel in sufficient quantities to enable the micro-organisms in the tubules to decalcify the dentine.

FIG. 2. Human upper lateral. Enamel etched with lactic acid on the labial surface. Incident light, × 8.

3. Experiments *in vitro* with the aim of sustaining the bacteria in the tubules by a diffusion of carbohydrate through opaque enamel have never succeeded.

4. A penetration of free acid from the bacterial plaque is also very improbable, as it is hard to understand how the dentine could be decalcified completely by acids passing first through only partly decalcified enamel.

5. In sections of carious teeth, the decalcification zone in the dentine always shows a clearly defined curved border, the softened dentine occupying a more or less spherical "cavity". This regular cavity form and the distinct borderline, so

FIG. 3. Ground section of human premolar. Caries of enamel and dentine. Transmitted light, × 40.

FIG. 4. Paraffin section of human premolar. Gram stain. Dentine caries. Only a few tubules contain micro-organisms. The decalcified area is sharply defined and distinctly spherical. Transmitted light, × 60.

well known from röntgenograms, are explained very unsatisfactory in most textbooks by correlating them with the progress of the micro-organisms in the tubules. Moreover, in many cases the decalcified dentine is only partially infected or even completely sterile, as was already discovered and described by MILLER (Fig. 4).

These facts and other considerations based on clinical experience have caused many research workers to drop MILLER's views altogether, and to search for another explanation of the carious process. The results were invariably of doubtful value, as the importance of acid production cannot be minimized without giving rise to new and still more puzzling problems. At present the literature on dental caries abounds with aetiologic factors, most of which are purely fantastic.

A thorough survey and analysis of all information has convinced the author that the acids formed in the plaque are an essential factor in dental caries but that they act indirectly, setting off some independent physico-chemical process that causes decalcification by lowering the pH *within* the tooth structures. A process of this kind has been described by DONNAN and coworkers in 1911. For a complete description of his work we must refer the reader to the literature. An outline of the principles involved is taken from TENDELOO (1952).

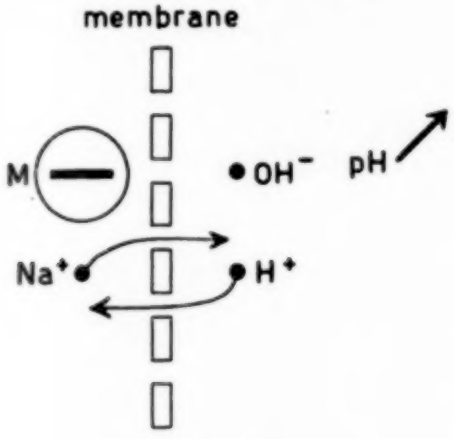


FIG. 5.

DONNAN studied the ionic concentrations in solutions separated by a semi-permeable membrane. When one of the solutions contains an ion large enough to be arrested by the membrane, interesting diffusion phenomena occur. For our purpose, the most important of these is the presence of large non-diffusing ions on one side of the membrane and their influence on the dissociation of pure water in the opposite compartment and on the distribution of its ions.

Let us consider a substance Na^+M^- dissolved and completely dissociated on the left side of the membrane. Under conditions as pictured in Fig. 5 the Na^+ ions can diffuse through the membrane to the right side, but they will only do so if the M^- ions go with them (which is impossible) or if an H^+ ion passes to the left in exchange for each Na^+ ion. DONNAN showed that this exchange actually takes place until a state of equilibrium is reached, which of course is influenced by the

concentration of Na^+ M^-. The right compartment then contains OH^- ions in excess of the remaining H^+ ions, in other words, its reaction has become alkaline, while the solution on the left side has turned acid.

Reverse conditions, viz. when the non-diffusing ion has a positive charge, will result in a low pH in the right compartment and a high reading on the left side. In this case (Fig. 6), the substance E^+ Cl^- providing the non-diffusing ion E^+, an exchange of Cl^- and OH^- ions will occur, again resulting in an equilibrium, dependent on the concentration of E^+ Cl^-. The right compartment then contains some HCl, or some other acid as the case may be. DONNAN has pointed out that in this way a pH as low as that of gastric juice may be attained. It must be kept in mind, that the low pH reading on the right side does not necessarily indicate the presence of large quantities of HCl. When however any of the ions concerned are continually removed as a result of some secondary chemical reaction in either

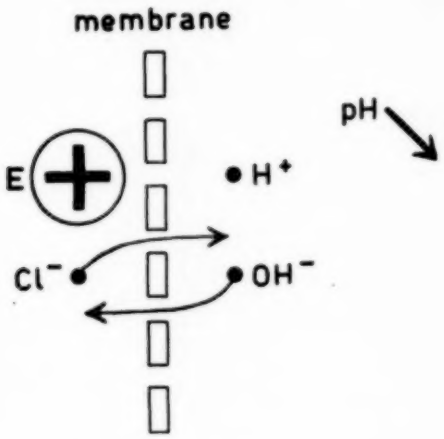


FIG. 6.

compartment, the transport of OH^- and Cl^- ions through the membrane will also be continuous. The possibilities outlined above will serve to show that under appropriate conditions ionic distribution causing a pronounced change of pH is possible and predictable as the result of membrane equilibria of the type described by DONNAN. In a number of experiments we have applied this theory to the problem of carious decalcification.

As enamel may be regarded as a water-containing gel of very dense structure, it will in its normal environment contain H^+ and OH^- ions equally distributed. Because of its density it will only admit small molecules and ions from the oral fluid, but it is quite impenetrable by macromolecules such as proteins. We may therefore consider the enamel surface as a membrane or barrier in the sense of

DONNAN's theory, and at the same time its mass as one of the "compartments", the other being the oral cavity. The fluid bathing the tooth surface may be a film of salivary mucin *or* the gelatinous contents of a bacterial plaque.

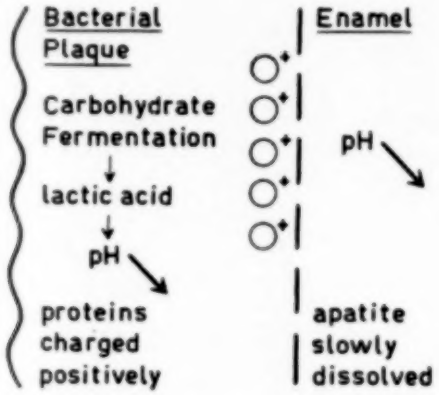


FIG. 7.

In the immediate proximity of the enamel surface we may assume the presence in solution of positively charged ions, too large to diffuse into the enamel (Fig. 7). A DONNAN ionic distribution will take place, resulting in a drop of the pH within

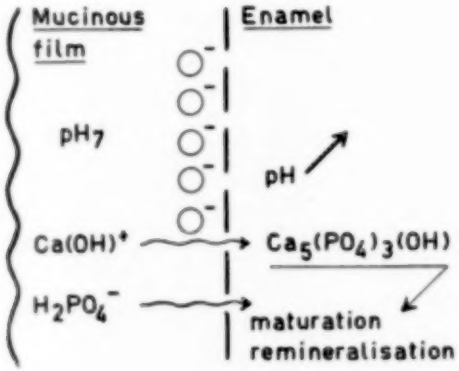


FIG. 8.

the enamel. The latter will "excrete" OH^- ions in exchange for any small anions present in the surrounding fluid. As an increasing excess of H^+ -ions develops within the enamel, we may suppose that the latter will be decalcified under conditions that are exactly opposite to decalcification by immersion of a tooth in an acid.

It has been shown that this particular kind of decalcification is actually responsible for the development *in vitro* of the characteristic "white spots" of initial enamel caries, and, in later stages, for the decalcification of the dentine as well. As the excess of H^+ ions will take part and be used in the ensuing process of decalcification the ion exchange will continue as long as positively charged ions are present on the enamel surface, or in other words, as long as predominantly positively charged macromolecules are present in true or colloidal solution.

The presence of negatively charged ions must give rise to the reverse situation, causing the pH in the enamel to rise slightly and thus exerting a stabilizing influence on the apatite structure (Fig. 8). Our experiments indicate that this situation actually occurs, the results pointing to salivary mucin as the active agent in enamel maturation and remineralization.

Vol. 6
1961

Perhaps the most important fact, that can be easily deduced from Fig. 5–8, is that the ionized macromolecules are not used up in any way. They need not even exist freely in a molecular solution, but can exert their influence as well or even better when forming a hydrated gel on the enamel surface (BOOY, 1956).

EXPERIMENTAL METHODS

All experiments were conducted at room temperature on extracted sound human teeth, except in those cases where carious teeth were selected for comparative purposes. In most cases the teeth used were upper and lower premolars that had been kept for some time, varying from a few days to several weeks, in 4% formol; the results did not differ in any way from those obtained with freshly extracted specimens. The teeth were cleaned with curettes, pumice and chalk, dried superficially and covered with paraffin by immersing them in a mixture of 90 parts paraffin of 60°C melting point and 10 parts beeswax, heated slightly above its melting point. The beeswax was added to render the paraffin less brittle. Windows were punched in the paraffin layer in order to expose one or more round areas of enamel to the influence of different solutions. After varying exposure times the teeth were cleaned and ground sections or paraffin sections were prepared through the centre of the former exposure window and through the tooth's axis. Three series of experiments were conducted.

In the first series the influence of acids was studied as to concentration and pH of different organic and mineral acids. In some cases the effect of different buffer mixtures was examined, because in the opinion of some authors the buffering action of the plaque constituents may be held responsible for the typical aspect of carious decalcification. The results of this series are compiled in Table 1.

All experiments with mineral acids (hydrochloric, sulphuric, and nitric acid) have been omitted, because these acids cause a rapid destruction of the ename that does not bear the slightest resemblance to caries. Buffer formulae were taken from LEHMANN (1928).

In a second series of experiments the effect of solutions of positively charged ionized macromolecules was studied. As most macromolecular compounds that form positive ions are very feeble bases, their soluble salts showing hydrolysis in solution, it was difficult to conduct an experiment at neutral pH. A solution of quinine

TABLE 1. SITUATION AFTER 30 DAYS

	pH Values								
	7	6·5	6	5·5	5	4·5	4	3·5	3
Aq. dist. + lactic acid	—	—	—	Ag	Ag	Ag	Bd	Cd	Cd
Buffer solution "Soloid" ph 7 + lactic acid	—	—	Ag	Ag	Bd	Cd	Cd	E	E
Buffer solution "Soloid" pH 4 + NaOH or lactic acid	—	—	Cg	Cd	Cd	Cd	Cd–D	E	E
Buffer solution Prim. -Sec. phosphate ("SÖRENSEN")	—	—	—	—	Ag				
SÖRENSEN buffer solution pH 7 + lactic acid	—	—	—	—	Bg	Bg	D	E	E
Buffer solution Sec. phosphatecitric acid (LEHMANN, p. 55)	—	—	—	Cd–D	D	D	D	E	E
Buffer solution K. monocitrate-borax (LEHMANN, p. 52)	—	Bg	Bd-D	Bd–D	D–E	E	E	Cd	
Buffer solution K. monocitrate-borax (LEHMANN, p. 52) + lactic acid	Bg	Bd	D	D	E	E	E	E	E

Index to symbols: A = very slight white spot, g = glossy surface, d = dull surface,
B = slight white spot,
C = white spot,
D = etched-out surface,
E = surface etched away, enamel changed into soft pappy mass,
F = surface layer intact, underlying enamel soft and pappy.

hydrochloric however was found to have a pH of 6·7 owing to the fact that only one of its two hydroxyl groups has been replaced by Cl^-. Its molecular weight is 324, and therefore it can be assumed that it will not enter sound enamel. A 3% solution in water at 37°C proved to decalcify enamel as expected. After 2 weeks the enamel showed all the characteristics of initial carious decalcification. A typical "white spot" had developed, extending to some depth into the enamel, without any break in the surface.

Further experiments were conducted with gelatin and gliadin solutions. Because of their typical protein structure they possess both basic and acid groups in watery solution, (mainly $—NH_3(OH)$ and —COOH). The H^+ ion is split off more easily than the OH^- ion, most pure proteins therefore possessing acid properties in water.

A solution of pure gelatin has a pH of 4·7 which is its isoelectric point. Addition of acid to the solution of isoelectric gelatin will slowly lower its pH, the gelatin forming a salt while behaving as a base (Fig. 9). The H^+ ions from the acid will

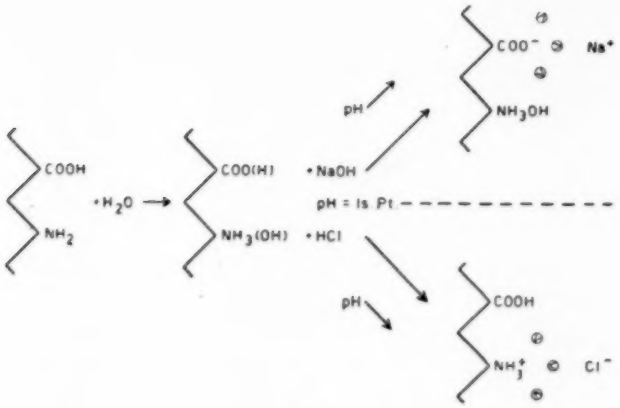


FIG. 9.

disappear as such, the pH falling only because of hydrolysis, until all positive valencies have been saturated with anions. The salt formed will be able to cause a DONNAN distribution of H^+ and OH^- ions as shown in Fig. 7, because the ionized protein has a positive charge. After addition of still more acid the solution will contain gelatin chloride and free acid.

Addition of base to the solution of isoelectric gelatin will cause a slow rise of pH, the protein now forming an anion and behaving as an acid. The same speculations apply to gliadin, a vegetable protein that has its isoelectric point at pH 6·8. Addition of lactic acid caused the formation of a precipitate; the sticky mass obtained after decanting the clear fluid was used in the same way as the acidified gelatin. A series of sterile containers were filled with solutions with different pH values, and a number of teeth, prepared as previously described, were immersed in each container so that the uncovered parts of the tooth surface were in direct contact with the solution. For all solutions distilled water was used with a little thymol added to prevent the growth of molds. A PYE pH meter was used for determining and controlling all pH values. After a certain length of time the teeth were cleaned and ground sections of about 100 μ prepared. Tables 2–4 represent some of the most important experiments.

TABLE 2

	pH Values										
	7	6·5	6	5·5	5	4·7	4·5	4	3·5	3	2
Gelatin 10%, NaOH or lactic acid 30%, 8 days	—	—	—	—	—	—	Bg	Cg	Cg	Cg	—
Gelatin 10%, NaOH or lactic acid 30%, 14 days	—	—	—	—	—	—	Ag	Bg	Cg	E	—
Gelatin 10%, lactic acid 30%, 21 days	—	—	—	—	—	Ag	Cg	Cg	Cg	F	E

For explanation of symbols see Table 1.

TABLE 3

	pH Values						
	7	6·8	6·5	6	5·5	5	4·5
Gliadin 10% NaOH or lactic acid 30% 30 days	—	—	—	Ag	Ag	Cd	Cd

For explanation of symbols see Table 1.

TABLE 4

No. days	Mean depth of white spot (mm)	No. days	Mean depth of white spot (mm)
5	0·08	75	0·47
10	0·12	80	0·51
15	0·15	85	0·54
20	0·18	90	0·60
25	0·21	95	0·67
30	0·26	100	0·74
35	0·28	105	0·76
40	0·33	110	0·81 DE
45	0·39	115	0·83
50	0·40	120	0·87 DE
55	0·41	125	0·99 DE
60	0·42	130	1·10 DE
65	0·42	135	1·01 DE
70	0·44	140	1·14 DE

The markings DE denote that in one or more of the specimens concerned the dentine-enamel junction was reached.

Gelatin 20% brought to pH=4 with 30% lactic.

In a third series of experiments the DONNAN phenomena caused by salivary mucin were studied. At the oral pH of about 7, mucin is negatively charged and probably present as its K^+ salt (RADSMA, 1954). As mucin has little or no basic groups, addition of only a little acetic acid to saliva will repress the dissociation of the carboxyl groups, the neutralized substance falling out from solution as a viscous whitish mass, which is readily soluble again in very diluted alkali. This procedure enabled us to obtain pure solutions of mucin of known concentration. Attempts to acidify these solutions so as to obtain positively charged mucin particles were of course unsuccessful. The mucin precipitated immediately, redissolving only after addition of an excess of mineral acid. The resulting solutions had the same effect on enamel as if they had contained acid only. From these results it may be concluded that strictly speaking mucin is not amphoteric like most other proteins. At its normal pH it is strongly negative, but a slight drop of the pH readily converts it to a neutral, unionized and therefore insoluble precipitate, that has no further part in any accompanying reaction. These findings agree with the work of OLDFELDT (1936) who found strongly acid properties in the case of salivary mucin (submaxillaris) with an isoelectric point as low as 2·5, which is in good agreement with the value of about 2·7 found by other workers. The isoionic point was somewhere at pH 3·4–3·45. A very characteristic finding for mucin was its very slight acid-binding capacity as compared to its capacity to bind base (1 : 5·3).

RESULTS

All teeth from the first series of experiments (Table 1), in which acids were used to attack the exposed tooth surface, showed a more or less pronounced etching and dissolution of the enamel after a varying length of time. Only in cases of very slight and superficial attack did the surface remain glossy during the first few weeks, but soon it became as dull as was the case with the stronger solutions. In all series sooner or later the enamel lesions passed through the same stages as described at the foot of the table. In the first experiment the unbuffered solutions of course required only very small additions of acid to obtain and maintain pH values of 6·5–4·5. Therefore only the solutions from pH 4 downward had sufficient strength to attack the enamel surface with visible results. The buffers chosen were all composed of substances of low molecular weight, and presumably unable to cause a DONNAN effect. In the buffered solutions the attack started immediately from pH 6 downward. Otherwise there was no difference from the unbuffered solutions. This proves that the buffering in itself has no effect on the course of acid decalcification, insofar that no better resemblance to carious decalcification could be obtained than with acids alone. A striking exception was found with the SÖRENSEN phosphate buffers, where no lesion occurred at pH 5·5 and over. It would seem that the presence of phosphate ions lowers the critical pH where attack may start. As for the remaining

pH values, once attacked the decalcifications showed the same characteristics as in the other specimens.

In the second series the results of the experiments with gelatin and gliadin confirmed our speculations regarding the posibility of a decalcification of the tooth substance by way of DONNAN membrane phenomena, as could already be expected from the aforementioned preliminary set-up with quinine hydrochloric.

Results are presented in Tables 2–4. Fig. 9 shows the general principles of these experiments. Part of a protein chain bearing an acid and a basic group is represented diagrammatically by the angular line. Depending on the presence and relative number of positive and negative groups per molecule a given protein has a specific isoelectric point. The watery solution of this protein therefore has a pH that corresponds with the specific isoelectric point of the protein. Addition of a base (e.g. NaOH) will result in the formation of the proteinate, the Na^+ ions remaining in solution, and the protein molecules bearing a number of negative charges at appropriate sites of the side groups. The free base disappears as such and is said to be buffered; it should be kept in mind that the process is in fact a salt being formed, and that it is not buffering in the strict sense. The pH rises because of hydrolysis, and the ionized protein obtains a water mantle, causing the solubility to increase and the solution to become more viscous. Addition of acid has the opposite effect as to the charge obtained, the protein now being charged positively at the sites of its aminogroups. The acid is "buffered", the pH slowly drops below the isoelectric point, and protein chloride, lactate, or another compound is formed according to the acid used. Again a water mantle forms around the ionized protein molecule, accompanied by increased solubility and viscosity.

When all negative groups have been saturated with base, or all positive groups with acid, the buffer capacity of the protein will be exhausted. If in the last mentioned case more acid is added, this will be present as free acid and cause the same lesions as in the first series of our experiments. In the case of salivary mucin however, which abounds with acid (—COOH) groups, basic groups lacking almost entirely, it is of course impossible to obtain a salt with an acid, except by a crude treatment with excess of mineral acid.

In the experiments now under consideration we used solutions of gelatin and gliadin. As has been explained above both substances can be given a positive or negative charge at will; the only difference is in their isoelectric points, which are at pH 4·7 and 6·8 respectively. The acid added was buffered, the important difference with the first series of experiments being that the buffering or salt-forming substance was non-diffusable. On the strength of this fact we could assume that a DONNAN effect would ensue. A positively charged gelatin or gliadin would tend to cause a drop of the pH within the enamel, while a negative charge will result in a basic reaction of the free water in the enamel structure.

Some experiments are summarized in Tables 2, 3, and 4, which only mention the macroscopic appearance of the enamel surface. Not included are the microscopic findings from the corresponding ground sections which will be dealt with separately. The most important conclusions that can already be drawn from these tables are the following. In conformity with our expectations, at all pH values above the isoelectric points (irrespective of their absolute numerical value) the teeth remained undamaged, even if these values were under pH 7. In our opinion this situation exists normally in the mouth, where the tooth surfaces are in contact with saliva, the mucinous film derived from this fluid being strongly negative at the normal oral pH.

Vol. 6
1961

Below the isoelectric point the macromolecules change their overall charge from predominantly negative to positive and the enamel is subjected to internal decalcification. As will be shown, this decalcification is identical to caries as far as the loss of calcium salts are concerned, both from the enamel and the dentine. As can be deduced from Tables 2 and 4 the best and quickest results are obtained when the gelatin concentration is high, and the pH is only slightly under its isoelectric point of 4·7. The drop of pH in the enamel depends on the gelatin concentration according to

$$[H^+] = \sqrt[3]{(K_w \cdot C_{gelatin})}$$

as computed by DONNAN, which explains the necessity of a high gelatin concentration. The acid-binding capacity of the concentrated gelatin gel should not be exceeded, solutions of pH 3·5 already showing marks of direct acid action. The most suitable pH at room temperature proved to be pH 4 or slightly over.

The histological aspects of the teeth decalcified under these optimal conditions are the following:

1. The exposed surfaces show a white discoloration which starts after a few days, sometimes even after a few hours.

2. The white spot keeps all surface characteristics of sound enamel, as could be affirmed by incident light microscopy and a replica technique described by PANTKE (1956).

3. In longitudinal and transverse ground sections the lesions show the same alternations as can be observed in dental caries, of course with the exception of infection, pigmentation and proteolysis. We studied these with incident and transmitted light, darkfield illumination, phase contrast and polarization microscopy (Fig. 10a, b, c).

4. When the lesion reaches the dentino-enamel junction, the dentine is decalcified in the form of a slowly widening spherical area, thereby progressively undermining the enamel (Fig. 11).

5. In most cases two transparent bands can be observed to extend from the decalcified zone to the pulp chamber, following the course of the dentinal tubules.

As in actual caries, these bands—in reality a tube of "sclerosed" dentine—enclose the "dead tract" described by FISH (Fig. 12). The cause of this transparency could not be ascertained with certainty, but it seemed to be part of the total picture, a "defence mechanism" of course being excluded absolutely.

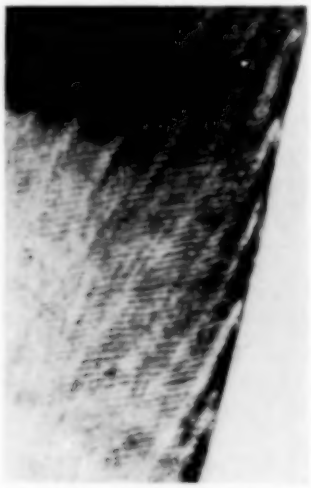


FIG. 10 (a). Ground section of human premolar. Artificial enamel caries. Gelatin 20% with lactic acid 30%, pH = 4. Transmitted light, × 360.

FIG. 10 (b). Ground section of human premolar. Artificial enamel caries. Gelatin 20% with lactic acid 30%, pH = 4. Incident light, × 15.

6. In some cases the undermined enamel shows a retrograde decalcification (Fig. 13).

Vol. 196

7. The border between totally decalcified and undecalcified dentine is rather abrupt and sharply defined (Fig. 11 and 13). When, however, calcium monophosphate is added from the start to the gelatin in excess, both areas blend evently into

FIG. 10 (c). Ground section of human premolar. Artificial enamel caries. Gelatin 20% with lactic acid 30%, pH = 4. Polarized light, total extinction, × 50.

FIG. 11. Ground section of human premolar. Artificial enamel caries. Gelatin 20% with lactic acid 30%, pH = 4. Transmitted light, × 30.

one another (Fig. 14). As in the first series of experiments, addition of phosphates strongly retarded decalcification in those experiments where the time factor was varied, and it lowered the critical pH in those series, where a descending scale of

pH values was prepared. As yet we are unable to offer an explanation of this phenomenon.

At the present time our observations on the activity of the gliadin solutions are limited to those given in Table 3, the development of lesions with involvement of

FIG. 12. Ground surface of human premolar. Artificial caries. Gelatin 20% with lactic acid 30%, pH = 3·5. Decalcified dentine stained with bromthymol blue, added to the mixture from the start. Incident light, × 10.

the dentine as shown in Fig. 12 taking from 1 to 2 years. However, from the results obtained already with gelatin as outlined above, confirmed by comparative studies with different microscopic techniques mentioned before, we have come to the

FIG. 13. Ground section of human premolar. Artificial caries Gelatin 20% with lactic acid 30%, pH = 4. Transmitted light, × 100.

conclusion that the presence of positively charged ionized macromolecules in solution, or as a hydrated gel on the enamel surface, is sufficient but necessary to create a DONNAN membrane effect, thereby decalcifying the enamel and successively the dentine in a way that bears the closest resemblance to actual caries.

In a recent experiment the effect was studied of adding a neutral salt to a standard decalcifying mixture of 25% gelatin brought to pH 4 with lactic acid. It was assumed that this addition of NaCl, in itself a harmless substance, would intensify the decalcification, as it could be expected that the Cl^- ion would diffuse better into sound enamel, being smaller and having a greater mobility and activity than the lactic acid ion, apart from its probably greater dissociation. As expected, the teeth from mixtures containing 1 or 2% NaCl showed a nearly doubled decalcification intensity as measured in depth of the white spot, and as compared to the controls without NaCl.

Vol. 6
1961

FIG. 14. Ground section of human premolar. Artificial caries. Gelatin 20% with lactic acid 30%, pH = 4. Transmitted light, × 20.

With greater amounts of NaCl the effect disappeared again, probably because the dissociation of the gelatin was suppressed by the higher NaCl concentrations. Perhaps the same situation exists in the bacterial plaque in the human mouth, where a definite concentration of Cl^- ions may be an important factor in the very first stages of enamel attack rather than the actual presence of lactic acid ions, taking into consideration the very narrow "spaces" that are present in normal enamel, and seem to remain so in the superficial layer during a considerable time before surface etching and cavitation occur.

In the third series of experiments the influence of negatively charged macromolecules was tested. Neither gliadin nor gelatin solutions at pH values above their isoelectric points proved to cause ill effects on the enamel, even if the actual pH readings were under 7. In the opinion of the author this is a result of the pH shift caused by the presence on the tooth surface of negatively charged particles, as

already represented in Fig. 5. Any small ions present in the fluid, when diffusing into the enamel, will find an alkaline reaction there. The saliva being saturated with calcium monophosphate at normal oral temperature and pH, the salt will precipitate after diffusing into the enamel, and the precipitate must pass via secondary phosphate to tertiary phosphate in the form of hydroxyapatite. Probably this is what happens in the mouth when a tooth surface attacked previously by initial caries is exposed to the cleansing action of mastication and to salivary flow, as is so often the case when a neighbouring tooth is removed. Evidently a DONNAN membrane effect of this kind may also form the basis of normal tooth mineralization and enamel maturation (Fig. 8).

DISCUSSION

In the opinion of the author the results described, when applied to the problem of dental caries provide a clear insight in the mechanism of carious decalcification. Initial caries of enamel, the so-called white spot, is the result of a ionic distribution of the DONNAN type, dependent on the presence and concentration on the tooth surface of positively charged ionized macromolecules, namely the proteins and their derivatives in the bacterial plaque, rather than the presence of free acid. Important factors are acid production rate, average isoelectric point and concentration of the proteins present, and the rate of clearance of all compounds concerned. The lactic acid formed in the plaque lowers the pH on the enamel surface only slightly, at the same time reacting with the proteins present from food debris, etc. If the pH drops sufficiently to cause a predominantly positive ionization of the soluble proteins present, a white spot will form, followed by the well-known other phenomena of carious decalcification.

The same DONNAN membrane laws serve to explain the dentine decalcification. As was proved by our experiments the dentine can be decalcified completely by a DONNAN effect. This proves that in actual caries the presence of micro-organisms in the tubuli is only secondary, and their activity is directed mainly to a proteolytic destruction of the remaining dentine matrix and TOMES' fibres.

When the tooth surface is not covered by a bacterial plaque, the situation and the ensuing DONNAN effect are exactly the opposite of the foregoing. A thin film of mucin is always present on the tooth surfaces that are freely exposed to the saliva. This film adheres rather strongly to the tooth, and when removed is quickly formed again. At the normal salivary pH of about 7 this substance is very strongly ionized, bearing negative charges and thus exerting a favourable influence on the state of mineralization of the hard tissues.

Experiments to establish the degree of ionization at pH 7 of individual samples of human salivary mucin are under way. Obviously at this pH a given mucin may carry a greater number of negative charges per molecule than some other sample.

Vol. 6
1961

Comparative measurements of this relative particle charge may prove to show better correlation with caries activity than has been obtained until now by simple pH measurements of whole saliva.

REFERENCES

BARTHELD, F. VON 1958. Decalcification in initial dental caries, *Tijdschr. Tandheelk.* **65**, 76.
BOOY, H. L. 1956. *Inleiding tot de Physische Biochemie*. (ed. 1). Haarlem, Boon.
DONNAN, F. G. 1925. *Chem. Rev.* **1**, 73.
DONNAN, F. G. 1932. *Kolloid Z.* **61**, 160.
DONNAN, F. G. 1934. *Z. phys. Chem.* A **168**, 369.
DONNAN, F. G. 1939. *J. Chem. Soc., Lond.* (1939), 707.
LEHMANN, G. 1928. *Die Wasserstoffionenmessung*. (ed. 1). Leipzig, Barth.
PANTKE, H. 1956. Die Replica-Technik. *Stoma* **2**.
RADSMA, W. 1954. *Inleiding tot de Physiologische Chemie* (ed. 1). Groningen, Wolters.
TENDELOO, H. J. C. 1952. *Kolloidchemie* (ed. 3). Gorinchem, Noorduijn.

Arch. Oral Biol., Special Supplement, Vol. 6, p. 304, 1961. Pergamon Press Ltd.

FACTORS INVOLVED IN REMINERALIZATION OF CARIOUS LESIONS

F. BRUDEVOLD, B. H. AMDUR and A. MESSER

Forsyth Dental Infirmary, 140 The Fenway, Boston 15, Mass., U.S.A.

Abstract—Although the role of the decalcified enamel matrix in remineralization of carious lesions is uncertain, our *in vitro* studies demonstrate that salivary sediment can act as matrix, and initiate calcification, and that apatite crystals can also catalyse crystal formation. Salivary sediments invariably calcified when they were exposed to calcifying solutions. The calcifiability was not destroyed by heating the sediment at 70°C for 30 min, nor was it dependent on the presence of viable micro-organisms. Mineral formation induced by hydroxy-apatite was greatly accelerated when traces of F (F 0·2 p.p.m.) were present in the calcifying solution. This effect of F was apparent in the pH range studied (pH 6·2–7·4). CaF_2 was not formed in these experiments, but F was rapidly removed from the solution and was probably deposited as fluor-hydroxyapatite.

The presence of Mg or carbonate in the calcifying solution retarded the rate of crystal formation. Small concentrations of F compensated the Mg effect.

It was concluded that the ionic species present in saliva and in tooth structure affect the process of remineralization and that the net effect of F as well as that of other ions is a sum total of complex interionic action.

Zusammenfassung—Obwohl die Rolle von entkalkter Zahnschmelzmatrix bei der Remineralisation kariöser Läsionen unbestimmt ist, zeigen unsere *in vitro*-Untersuchungen, daß das Speichelsediment als Matrix dienen kann und die Verkalkung in Gang setzt und daß auch Apatitkristalle die Kristallbildung katalysieren können. Speichelsediment verkalkt immer, wenn es entsprechenden verkalkenden Lösungen ausgesetzt wird. Die Verkalkungsfähigkeit wurde durch 30-minütiges Erhitzen des Sedimentes auf 70° nicht zerstört; sie war auch nicht abhängig von der Anwesenheit lebender Mikroorganismen. Die Mineralbildung durch Hydroxylapatit wurde stark beschleunigt, wenn Spuren von F (F = 0,2 p.p.m.) sich in der Lösung befanden. Diese Wirkung von F war im untersuchten pH-Bereich (pH zwischen 6,2–7,4) offensichtlich. CaF_2 wurde in diesen Experimenten nicht gebildet, aber F verschwand schnell aus der Lösung und wurde wahrscheinlich als Fluorhydroxylapatit ausgefällt. Die Anwesenheit von Mg oder Carbonat in der Verkalkungslösung verzögerte die Geschwindigkeit der Kristallbildung. Niedrige F-Konzentrationen glichen den Mg-Effekt aus.

Es wurde daraus geschlossen, daß die Ionenarten, die im Speichel und in den Zahnstrukturen vorhanden sind, den Prozess der Remineralisation beeinflussen und daß der Effekt von Fluor und anderen Ionen in dem komplexen interionischen Geschehen zur Auswirkung kommt.

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 305–314, 1961. Pergamon Press Ltd.

THE BIOCHEMISTRY OF ENAMEL IN RELATION TO CARIES-RESISTANCE

G. N. JENKINS

University of Durham, Newcastle upon Tyne, England

Abstract—It might be expected that the composition of enamel would affect susceptibility to caries but this is difficult to investigate for several reasons. In particular, since caries begins at small imperfections in the enamel, it may be that it is the composition at these sites, rather than of the enamel as a whole, which affects the resistance of the tooth. No differences have been established in human enamel related to caries-resistance (except the fluoride content in high fluoride areas) although a number of suggestions have been made. Animal experiments suggest that the P/CO_3 ratio, the carbohydrate content or the presence of certain trace elements might be related to caries but these points have not been thoroughly investigated in man.

Zusammenfassung—Man könnte erwarten, daß die Zusammensetzung des Zahnschmelzes die Kariesanfälligkeit beeinflussen würde, aber es ist schwierig, darüber Untersuchungen anzustellen Insbesondere, da die Karies an kleinen, minderwertigen Stellen des Zahnschmelzes beginnt und die Zusammensetzung an diesen Stellen für die Resistenz des Zahnes eine größere Rolle zu spielen scheint als der Zahnschmelz als Ganzes. Es wurden bisher keine Unterschiede im Zahnschmelz bezüglich der Kariesresistenz gefunden (ausgenommen der hohe Fluorgehalt in Fluorgebieten), obwohl hierüber eine Anzahl Vermutungen geäußert worden ist. Tierversuche legen es nahe, daß der P/CO_3-Quotient, der Kohlenhydratgehalt oder das Vorhandensein gewisser Spurenelemente in Beziehung zur Karies stehen könnten. Beim Menschen wurde bisher keiner dieser Faktoren gründlich untersucht.

INTRODUCTION

AT FIRST SIGHT one very obvious approach to the caries problem and in particular to the question of why some people are more prone to caries than others, would be to investigate the composition of enamel in relation to its caries-resistance. On further consideration, however, this approach suffers from several disadvantages. In the first place, what can be regarded as caries-prone or caries-resistant enamel? Until caries has actually begun, and produced chemical changes, there is no means of knowing whether a particular tooth is caries-prone. Likewise, if one takes a caries-free-tooth there is no guarantee that it would not have become carious had it remained in the mouth for a longer time. It has been usual to compare the surviving tissues of a carious tooth with those of a tooth which, up to the time of extraction, had resisted caries. But there is the possibility that the products of the carious process may diffuse into the otherwise sound part of the tooth, thus altering its composition.

Alternatively, it cannot be necessarily assumed that the whole of the enamel would have the same composition and the surviving part may not show the difference in composition which caused the decayed part to have a low resistance to caries.

This point leads to another difficulty inherent in relating chemical structure to caries-resistance which has not always been considered. The enamel surface is now known to differ in composition from the enamel as a whole (JENKINS and SPEIRS, 1953; BRUDEVOLD, GARDNER and SMITH, 1956). Since caries begins on the surface, it might be expected that the surface composition would be most closely related to caries-resistance. The recent work of DARLING (1956, 1958), in showing that caries begins at small imperfections in the enamel surface, raises the possibility that differences of composition at these small points or immediately inside them might suffice to influence the resistance to a carious attack. If the composition of enamel, and perhaps dentine (BRADFORD, 1960), affects the rate of progress of caries, rather than the resistance to its initiation, then highly localized differences in composition would, of course, be less important.

It must be emphasized that the composition of the tooth could never be considered as the only factor involved in its caries-susceptibility. The question is rather how important is the composition compared with the morphological form, and spacing, of the teeth, the nature of the diet and eating habits, the rate of flow, the buffering and antibacterial power and other properties of saliva.

GROUNDS FOR BELIEVING THAT ENAMEL COMPOSITION IS RELATED TO CARIES RESISTANCE

There are at least four grounds for believing that the structure of teeth is related to caries resistance even though, for the reasons mentioned above, it may be difficult or even impossible to establish the details.

(1) The well-known work of SOGNNAES (1948a) suggested that the "war-time diet" effect involved, at least partly, an increased resistance of the tooth to account for the apparent time-lag in the fall in caries. There is now some doubt about whether the time-lag was real as PARFITT (1954) has pointed out that it would take several years for a change in rate of caries to influence the total cumulative caries score. This is particularly true when, as in this case, the data studied is somewhat crude and was not collected originally for research purposes. But there is also evidence in some teeth of an increased caries-resistance which remained even after war conditions had ceased (TOVERUD, 1957; HADJIMARKOS, 1960).

(2) The animal experiments carried out in an attempt to explain the apparent time-lag did show quite convincingly that caries-resistance in rats, mice, hamsters and monkeys could be influenced by both pre- and post-natal diet (SOGNNAES, 1948b). Although these results are of great interest in themselves, there is no proof that they provide the real explanation of the wartime diet effect.

(3) The work of SOBEL *et al.* (1960) has shown an effect in rats of the Ca/Po_4 ratio of the diet on the composition of enamel and the possible resistance to caries.

(4) The final approach which suggests that enamel structure is a factor in caries is the finding in animals that the environment of the teeth shortly after eruption can influence their resistance to caries (VOLKER, 1951). Thus, if a cariogenic diet is given to hamsters 8–12 days old, more caries develops during a constant period than if the cariogenic diet is withheld until the age of 45 days (FINN, KLAPPER and VOLKER, 1955). FANNING, SHAW and SOGNNAES (1953) have shown in rats that contact with saliva is necessary for this post-eruptive increase in caries-resistance, but the active substances and the nature of the changes in enamel composition which presumably occur have not been identified. ADLER (1957a) has presented clinical evidence for a post-eruptive increase in resistance of human teeth, and WEAVER (1944) gave reasons why water-borne fluoride might partly act just after eruption.

It might be speculated that a deposition of calcium and phosphate occurs on the enamel, perhaps sealing up some of the enamel imperfections at which caries begins. This may also be linked with the work of Knappwost (1951), supported by different types of experiments by PIGMAN, KOULOURIDES and NEWBRUN (1960) and BRUDEVOLD, MESSER and AMDUR (1961), suggesting that fluoride may increase the precipitation of calcium phosphate on the tooth surface.

Vol. 6
1961

DIFFERENCES IN THE INORGANIC FRACTION OF ENAMEL

The early literature contains suggestions of differences in composition between sound and carious teeth but the results are of limited value as whole teeth, or roots and crowns, were usually analysed rather than enamel and dentine. Precautions for excluding dental tissues altered as a result of caries were either unstated or inadequate.

GASSMAN (1921) reported that third molars (which he assumed to be more susceptible to caries than other teeth) contained excess calcium and carbonate, i.e. were deficient in phosphate. Other earlier results suggested that the magnesium of enamel from carious teeth was higher than in sound enamel (HOWE, 1926; KAUSHANSKY, 1932; ULRICH, 1925). MURRAY and BOWES (1936) found no differences between the calcium, phosphorus or total ash of enamel from sound and carious teeth but the carbonate was higher and the Mg lower in the carious samples.

ARMSTRONG and BREKHUS (1938) who analysed a small number of samples of enamel from sound and carious teeth reported no correlation between composition and susceptibility to caries (in Ca, P, Mg or CO_2). One set of their figures does show a difference in the Mg content, however (0·23 per cent for sound enamel, 0·32 per cent for carious). RITCHIE (1961) states that feeding magnesium phosphate to a group of 200 patients of varied ages haves reduced caries incidence. Analyses of the enamel surface from some of the teeth have suggested that magnesium may become attached to the surface where he thinks it may act as a mobile reserve

protecting the enamel. In a few teeth from the controls (not fed the salts) the enamel analyses suggested that the surface magnesium is higher in the enamel of caries-free subjects compared with caries-prone, but the number of analyses published is far too small, and the matching of the ages of the teeth is inadequate, to establish this point.

PICARD and CARTIER (1952) analysed the enamel and dentine from 120 human permanent teeth and found no differences in calcium, phosphate or ash in the enamel whether the teeth were sound or with superficial or deep cavities. Similarly negative results were obtained by OCKERSE (1949).

In animals, contradictory results have been obtained on the effect of feeding high sugar, caries-producing diets on the minerals of teeth. HARTLES (1951) reported that a 67 per cent sugar diet increased the calcium and phosphate concentrations of enamel and dentine but this was not confirmed by HALDI *et al.* (1955) who found no differences in any constituent. CREMER *et al.* (1953) analysed the whole teeth of rats fed for three generations on a refined, caries-producing diet containing 63 per cent sugar along with controls on a similar diet with only 14 per cent sugar. The only significant difference was a lower magnesium content (0·32 per cent) in the high sugar group (controls had 0·44 per cent).

It may be concluded that there is no detectable difference between the calcium and phosporus contents of whole enamel from sound and carious human teeth but that the CO_2 has been stated to be higher in carious-susceptible enamel (MURRAY and BOWES, 1936) and there are contradictory statements about magnesium.

FLUORIDE

It is now quite clear that the fluoride concentration of enamel is related to the fluoride ingestion which, in most people, means the fluoride level of the water supply and is, therefore, related to the high caries resistance of teeth formed in "high fluoride" areas. However, in addition to this well-established fact suggestions have been made that the fluoride concentration of enamel is related to variations in caries-resistance even in "low fluoride" areas. Thus, ARMSTRONG and BREKHUS (1938) stated that enamel from non-carious teeth contained an average of 0·0111 per cent fluoride whereas the surviving enamel from carious teeth averaged only 0·0069 per cent. These results were highly significant but have not been confirmed by others on larger groups of teeth (MCCLURE 1950; MACCLURE and LIKINS, 1951) and remain unexplained. Nevertheless, the fluoride of the surface enamel from carious and non-carious teeth has not been investigated and might be related to caries without altering perceptibly the total F of enamel.†*

* Since this paper was read, Nikiforuk (Caries Symposium, Zurich 1961, Huber, Berne p. 62) has published data which tentatively support the conclusion that the surface fluoride of non-carious enamel is higher (136 p.p.m. ± 20·6) than for carious enamel (83 p.p.m. ± 13·0) in residents of Toronto (F of water 0·1 p.p.m.).

Analyses of teeth from various parts of the world where caries is known to be low (India, Iran and the Canadian Arctic) have also shown that the fluoride of enamel and dentine was significantly higher than in teeth from Americans (SHAW, GUPTA and MEYER, 1956; SHAW, RESNICK and SWEENEY, 1959). It is impossible to say whether this difference arose from a higher fluoride intake (the intake was not known) or from other factors which influence fluoride absorption, such as low calcium intake or general malnutrition. This is an interesting point which deserves more investigation.

EFFECT OF CO_3/PO_4 RATIO

SOBEL and HANOK (1948) have shown in cotton rats that by feeding diets with varying Ca/PO_4 ratios it is possible to affect the CO_3/PO_4 ratio (but not the Ca/PO_4 ratio) of the bones and teeth. A low Ca/PO_4 ratio of the diet (i.e. a diet high in PO_4) resulted in a low CO_3/PO_4 ratio in the teeth enamel and dentine. Since several workers have found that carbonate is dissolved out from a tooth in preference to phosphate it might be predicted that a high CO_3/PO_4 ratio in enamel would increase its solubility and thus favour caries. Experiments did indeed show that cotton rats fed on a non-cariogenic diet with a low Ca/PO_4 ratio during the formation of their teeth and then put on to a cariogenic diet with a similar Ca/PO_4 ratio for 12–14 weeks showed both a smaller number and size of carious lesions compared with similar animals on a high Ca/PO_4 ratio during tooth formation (SOBEL, 1955; SOBEL *et al.*, 1960).

The recent finding that a high level of phosphate in a diet reduces its cariogenicity complicates the interpretation of this experiment. There is no proof that the (presumably local) effect of the high phosphate of the diet which promoted the low CO_3/PO_4 and the low caries rate did not account for it. To prove the importance of tooth composition on caries resistance, it would be necessary to feed the diets with different Ca/PO_4 ratios to groups of animals during the formation of their teeth and then feed a cariogenic diet with a normal Ca/PO_4 ratio to both groups. It must also be emphasized that the Ca/PO_4 ratios in the diets of SOBEL *et al.* were quite outside the variations found in human diets.

Few analyses of the CO_3/PO_4 of human teeth have been carried out but BOWES and MURRAY (1936) analysed enamel from teeth with varying degrees of hypoplasia. They showed that the percentage of CO_2 increased as the severity of hypoplasia increased. If it be accepted that hypoplasia reduces the caries-resistance of a tooth, these results support SOBEL's conclusion. As mentioned earlier, MURRAY and BOWES (1936) reported also that enamel from carious teeth (without hypoplasia) had a higher carbonate and the same phosphate (i.e. high CO_3/PO_4 ratio) than enamel from non-carious teeth.

The general question of vitamin D, hypoplasia and caries will not be discussed here since this is probably more a question of morphology than biochemistry.

Vol. 6
1961

TRACE ELEMENTS

SOGNNAES and SHAW (1954) investigated the nature of the substance(s) present in natural diets which reduced the caries-susceptibility of animals when fed during the period of formation of the teeth. The fact that, in the original experiments of SOGNNAES, the caries of the offspring was affected when the animals were fed the natural or synthetic diets for some time before pregnancy, strongly suggested that trace elements were involved. It is almost inconceivable that changes in the major constituents of the diet could have pre-natal effects. SOGNNAES and SHAW found that the ash obtained by heating the natural diet to 450°C had an effect similar to, but not quite as great as, that of the natural diet and had an additional effect in reducing caries when fed prenatally. These facts suggest that some mineral substance, presumably a trace element, was entering the enamel and increasing its caries resistance. A qualitative analysis of the ash has been published but it is not known which of the many trace elements may be responsible for the action on caries. The trace elements of enamel formed on the natural, refined or refined plus ash diets have not been reported.

In addition to fluoride, some other trace elements have been implicated in caries. Vanadium (GEYER, 1953), molybdenum (ADLER and STRAUB, 1953; ADLER, 1957b; BÜTTNER, 1961) and manganese (STEPHAN and HARRIS, 1953; MÜNCH, 1958) have been stated to reduce caries and selenium to increase it (TANK and STORVICK, 1960; BÜTTNER, 1961). On the other hand, negative results have been reported on rat caries for vanadium and manganese (BÜTTNER, 1961). It is of interest that the ash of the diet prepared by SOGNNAES and SHAW contained fluoride, vanadium and molybdenum although the two latter elements were only detected qualitatively.

The concentration in human enamel of these trace elements in relation to caries does not seem to have been adequately studied, but MEULEN (1955) reported that the molybdenum concentration in the ash of non-carious permanent teeth was 0·55 p.p.m. but only 0·03 p.p.m. in carious teeth.

Molybdenum has been found to increase the absorption and retention of fluoride in rats (CRANE, 1960; STOOKEY and MUHLER, 1960) although BÜTTNER (1961) could not confirm this. ADLER and STRAUB (1953) found that the caries incidence in certain towns in Hungary was lower than might have been expected from the fluoride of the water supply, although molybdenum was higher than usual. The effect of molybdenum in reducing caries was confirmed in rats fed on a coarse corn diet and receiving 0·1 p.p.m. of molybdenum in their water. It would be interesting to know the fluoride and molybdenum contents of the enamel from residents of the Hungarian towns studied by ADLER and STRAUB.

In view of the evidence that the war-time diet effect on caries involved changes in the structure of teeth, an analysis was undertaken of the enamel of a small number of teeth formed before and during the war (JENKINS and SPEIRS, 1954). It was hoped

that this might reveal the nature of the dietary factor which had appeared to increase the resistance of the teeth, although, as the number of teeth was small (forty-nine prewar and forty-eight wartime) it was realized that only fairly large differences could be established.

Enamel separated by the Manly and Hodge method was analysed for: fluoride, CO_3/PO_4 ratio, carbohydrate and trace elements (qualitative). Enamel specimens ground off teeth with a bur were used for spectrographic examination for trace elements. In addition, the solubility in buffer at pH 4·0 of "windows" in the wax-covered enamel of the intact teeth were compared.

No differences were established, but slight non-significant trends were found towards reduced solubility and lower CO_3/PO_4 in the teeth formed during the war.

ol. 6
961

ENAMEL PROTEIN

Of two groups of workers who have studied the nitrogen content of enamel, one concluded that it did not differ in caries-prone or caries-resistant teeth (MURRAY and BOWES, 1936) but the other found that caries-susceptible teeth contained less nitrogen but the difference was of uncertain significance (DEAKINS and VOLKER, 1941). A careful investigation by HESS and LEE (1954) of the amino acid concentrations in the mixed proteins from the healthy enamel of seven groups of five carious molars when compared with similar figures referring to enamel from an unspecified number of caries-resistant teeth did show some marked differences. Methionine, hydroxy-proline and tryptophane were lower and threonine, alanine, tyrosine and leucine were higher in the non-carious although the percentage of other amino acids were within experimental error. Every care seemed to be taken to avoid contaminants (always a serious difficulty in dealing with enamel protein) and in excluding enamel which had been altered as a result of caries. HESS and LEE made no attempt to inter-pret these results, which do not seem to have been reinvestigated by other workers, but one might speculate that, if confirmed, they indicate that the various proteins of enamel are present in different proportions in carious and non-carious teeth. LITTLE, (1961) has suggested, from electron micrographs of sections of teeth with variations in caries resistance, that the protein in the prism core is more soluble in the caries-prone teeth. The groups of teeth compared included some from a high F area (5–15 p.p.m.) and from a caries-free population of 1800 years ago as well as from caries-prone and caries-susceptible subjects from the general population.

More work is urgently needed on the nature of the soluble protein of enamel and of the factors which influence its formation. Since DARLING and MORTIMER (1959) have suggested that removal of the soluble protein may be the first stage of caries (although their work has not yet been confirmed) more information about this material might provide new approaches to caries prevention. For example, if methods could be found of reducing its solubility or preventing its formation the resistance of the tooth could perhaps be increased.

OTHER ORGANIC CONSTITUENTS

Several lines of work have suggested that other organic constituents of enamel might be related to caries-resistance.

EGYEDI (1953) studied the carbohydrate concentration of teeth of natives of the Netherlands (high caries incidence) with those from Indonesia (low caries). He stated that the carbohydrate, as measured by the anthrone method, was higher in the enamel from the Dutch teeth and suggested that a high carbohydrate concentration favoured caries by providing nutrients for invading bacteria. STACK (1956) found no correlation between the carbohydrate of enamel and caries experience in British teeth.

As already mentioned, SOGNNAES found that the feeding of high sugar, refined diets to animals during tooth development and prenatally increased the susceptibility to caries. Although SOGNNAES himself never stated that the high sugar level of the diet was responsible, but suspected a low level of trace elements resulting from the refining of the dietary constituents, other workers (including EGYEDI) have sometimes assumed that the high sugar concentration was responsible.

Two feeding experiments on animals have been interpreted as supporting the idea that the carbohydrate of the diet during tooth formation influences the caries-resistance of the tooth which could also provide an alternative or additional explanation for the results of SOGNNAES's experiments on the prenatal feeding of natural and refined diets.

STEINMAN and HALEY (1957) carried out a number of experiments in which various carbohydrates (20% solutions) were administered by dropping pipette to groups of new-born rats throughout the period of suckling. In one experiment a control group receiving tap water was included. The animals were then fed a cariogenic diet. The results showed that the sugars, and also starch, when received by rats during tooth formation greatly increased caries susceptibility. The weights of the animals were not affected by this procedure (except in the starch group) from which it is concluded that the milk intake was not different in the different groups and the caries-susceptibility could only have been affected by higher carbohydrate intake.

BUXBAUM *et al.* (1958) compared the caries score and glycoprotein content of the teeth of rats fed either high sugar or natural diets similar to those by SOGNNAES. The results were interpreted as showing that both caries susceptibility and the glycoprotein content of the teeth were greatly increased by the high sugar diet. Unfortunately, this experiment is inconclusive because the whole teeth and not the enamel were analysed for glycoprotein. The analyses were carried out on the carious teeth and although they were washed and gross food debris was probably removed it may have remained in deep cavities. Also, in view of the finding of STACK (1954) that the concentration of carbohydrate in chalky enamel may be up to

twelve times higher than in normal enamel, the rise observed by BUXBAUM *et al.* may have followed the caries. The same criticism may apply to EGYEDI's results on Dutch and Indonesian teeth.

SUMMARY AND CONCLUSION

Apart from the fluoride concentration, no constituent of enamel is definitely known to be related to caries resistance in man. Many experiments have suggested possible differences, however, and more work is especially desirable on human teeth, with proper precautions against artefact, on the following:

(1) Concentration of magnesium and various trace elements, especially on the enamel surface;

(2) Carbonate concentration and the CO_3/PO_4 ratio;

(3) Changes occurring during the post-eruptive maturation of enamel;

(4) Qualitative differences in enamel protein;

(5) Quantitative differences in carbohydrate.

Acknowledgement — The author wishes to thank Professor P. ADLER, Dr. W. BÜTTNER and Professor H. MÜHLEMANN for help in tracing references in certain European journals.

REFERENCES

ADLER, P. 1957a. *Dtsch. zahnärztl. Z.* **12**, 12.

ADLER, P. 1957b. ORCA Proc. 4th Congress, p. 48.

ADLER, P. and STRAUB, J. 1953. *Acta med., Acad. Sci. Hung.* **4**, 221.

ARMSTRONG, W. D. and BREKHUS, P. J. 1938. *J. dent. Res.* **17**, 27.

BOWES, J. H. and MURRAY, M. M. 1936. *Biochem. J.* **30**, 977.

BRADFORD, E. W. 1960. *Brit. dent. J.* **109**, 387.

BRUDEVOLD, F., GARDNER, D. E. and SMITH, F. A. 1956. *J. dent. Res.* **35**, 420.

BRUDEVOLD F., MESSER, A. and AMDUR, B. H. 1961. ORCA Proc. 8th Congress.

BÜTTNER, W. 1961. ORCA Proc. 8th Congress.

BUXBAUM, J. D., KOHN, H., PROUTT, L. H. and OSTER, R. H. 1957. *J. dent. Res.* **36**, 173.

CRANE, D. B. 1960. *J. dent. Res.* **39**, 704.

CREMER, H.-D., BÜTTNER, W., DITTMANN, G. and VOELKER, W. 1953. *Biochem. Z.* **324**, 83.

DARLING, A. I. 1956. *Brit. dent. J.* **101**, 289, 329.

DARLING, A. I. 1958. *Brit. dent. J.* **105**, 119.

DARLING. A. I. and MORTIMER, K. V. 1959. *J. dent. Res.* **38**, 1226.

DEAKINS, M. and VOLKER, J. F. 1941. *J. dent. Res.* **20**, 117.

EGYEDI, H. 1953. *Dent. Items* **75**, 111.

FANNING, R. J., SHAW, J. H. and SOGNNAES, R. F. 1953. *J. Amer. dent. Ass.* **49**, 688.

FINN, S. B., KLAPPER, C. E. and VOLKER, J. F. 1955. In: *Advances in Experimental Caries Research* (Ed. by SOGNNAES, R. F.). Washington.

GASSMANN, 1921. *Beitrag zum Studium des chemischen Aufbaues des Knochen und Zahngewebes.* Bern.

GEYER, C. F. 1953. *J. dent. Res.* **32**, 590.

HADJIMARKOS, D. M. 1960. *J. dent. Res.* **39**, 590.

HALDI, J., WYNN, W., LAW, M. L. and BENTLEY, K. D. 1955. *J. Nutr.* **57**, 215.

HARTLES, R. L. 1951. *Biochem. J.* **48**, 245.

HESS, W. C. and LEE, C. Y. 1954. *J. dent. Res.* **33**, 62.

HOWE, P. R. 1926. *Dent. Cosmos* **68**, 1021.
JENKINS, G. N. and SPEIRS, R. L. 1953. *J. Physiol.* **121**, 21P.
JENKINS, G. N. and SPEIRS, R. L. 1954. *J. dent. Res.* **33**, 736.
KAUSHANSKY, L. I. 1932. *Dent. Cosmos* **174**, 468.
KNAPPWOST, A. 1951. *Z. Electrochem.* **55**, 586.
LITTLE, K. 1961. Proc. I.A.D.R. (British Section). In press.
MCCLURE, F. J. 1948. *J. dent. Res.* 27, 287 .
MCCLURE, F. J. and LIKINS, R. C. 1951. *J. dent. Res.* **30**, 172.
MEULEN, T. (1955). Handelingen van het 25 Nederland'sche Natuuren Geneeskundig Congress, p. 228.
MÜNCH, J. 1958. ORCA Proc. 5th Congress, p. 284.
MURRAY, M. M. and BOWES, J. H. 1936. *Brit. dent. J.* **61**, 473.
OCKERSE, T. 1949. *Dental Caries, Clinical and Experimental Investigation.* Dept. of Health, Pretoria.
PARFITT, G. J. 1954. *Brit. dent. J.* **97**, 235.
PICARD, J. and CARTIER, A. 1952. *C. R. Soc. Biol., Paris* **146**, 1488.
PIGMAN, W., KOULOURIDES, T. and NEWBRUN, E. 1960. *J. dent. Res.* **39**, 1117.
RITCHIE, D. B. 1961. *Nature, Lond.* **190**, 458.
SHAW, J. H., GUPTA, O. P. MEYER, M. E. 1956. *Amer. J. clin. Nutr.* **4**, 259.
SHAW, J. H., RESNICK, J. B. and SWEENEY, E. A. 1959. *J. dent. Res.* 38, 129.
SOBEL, A. E. 1955. *Ann. N. Y. Acad. Sci.* **60**, 713.
SOBEL, A. E. and HANOK, A. 1948. *J. biol. Chem.* **176**, 1103.
SOBEL, A. E., SHAW, J. H., HANOK, A. and NOBEL, S. 1960. *J. dent. Res.* **39**, 462.
SOGNNAES, R. F. 1948a. *Amer. J. Dis. Child.* **75**, 792.
SOGNNAES, R. F. 1948b. *J. Amer. dent. Ass.* **37**, 676.
SOGNNAES, R. F. and SHAW, J. H. 1954. *J. Nutr.* **53**, 195.
STACK, M. V. 1954. *Brit. dent. J.* **96**, 73.
STACK, M. V. 1956. *J. dent. Res.* **35**, 966.
STEINMAN, R. R. and HALEY, M. I. 1957. *J. dent. Res.* **36**, 532.
STEPHAN, R. M. and HARRIS, M. R. 1953. *J. dent. Res.* **32**, 708.
STOOKEY, G. K. and MUHLER, J. C. 1960. *J. dent. Res.* **39**, 671.
TANK, G. and STORVICK, C. A. 1960. *J. dent. Res.* **39**, 473.
TOVERUD, G. 1957. *Millbank Mem. Fd Quart.* **37**.
ULRICH, J. L. 1925. *Dent. Cosmos* **67**, 1204.
VOLKER, J. F. 1951. *J. dent. Res.* **30**, 484.
WEAVER, R. 1944. *Brit. dent. J.* **77**, 185.

Arch. Oral Biol., Special Supplement, Vol. 6, pp. 315–316, 1961. Pergamon Press Ltd.

RECENT ADVANCES IN DENTAL RESEARCH IN THE U.S.A.

F. A. ARNOLD, JR.

Director, National Institute of Dental Research

National Institutes of Health, Department of Health, Education and Welfare, Bethesda 14, Md., U.S.A.

Vol. 6
1961

Abstract—The National Institute of Dental Research dedicated its modern, 4 million dollar laboratory building, May 1961, on the grounds of the National Institutes of Health in Bethesda, Maryland. This represents the culmination of 13 years of steady growth and expansion of biologic research in dentistry, which had its beginning in 1948 when the Institute was established by Congress. In these few years the Institute has grown from a staff of less than a dozen members to its present complement of over 200 scientists and technicians with an annual appropriation of over $15 million. Also during this period the Institute's vital extramural program, under which grants are awarded to non-Federal institutions for research and training on oral diseases, has expanded from an annual $200,000 allotment supporting five grants, to an allotment of over $10 million supporting over 400 research grants and thirty training grants at some 100 institutions in all areas of the United States and several foreign countries. With this growth has come maturity and diversity in research into the causes and prevention of oral diseases–diversity into untried areas which only a few years ago were horizons for future experimentation.

Scientific breakthroughs, such as fluoridation, in the control of dental caries do not obscure the fact that the cause of this disease is still unknown. For this reason, the promising new lead from findings in animals that caries is a transmissible disease of bacterial origin, is being vigorously pursued. These findings carry widespread significance in the field of oral health because they explain such phenomena as resistance and susceptibility to caries, formerly attributed entirely to genetic factors and to systemic developmental defects induced by diet and nutrition. Their application to clinical studies carries implications of a possible immunologic or chemotherapeutic prevention of dental decay.

Biochemical studies of the cariostatic effects of mineral phosphates in the diet of animals have directed attention to the chemical reactivity of tooth surfaces. Other considerations include the nutrition of the oral flora and their metabolic products which may undergo significant change in the presence of freely available phosphate ions.

Progress in biologic research underscores the need for increased studies on periodontal disease, looming larger as a major cause of tooth loss in the United States. In consideration of our expanding population, of senior citizens, new research studies of ageing oral tissues will be encouraged and supported.

The Institute, through its grants and aid program, continues to increase the support of dental research projects particularly in the broad fields of periodontal

disease, oral congenital anomalies, and dental caries. Training centres for graduate studies now exist in nearly thirty institutions in the United States and are increasing rapidly. From these centres will come a new generation of dental scientists and teachers trained for biologic research and oriented to the prevention of oral disease.

Zusammenfassung—Das National Institute of Dental Research übergab im vergangenen Mai das moderne, 4 Millionen Dollar Laboratoriumsgebäude, das auf dem Anwesen des National Institute of Health in Bethesda, Maryland, steht. Das stellt einen Höhepunkt des über 13 Jahre sich erstreckenden, ständigen Wachstums und der Erweiterung biologischer Forschung in der Zahnheilkunde dar, das 1948 begann, als das Institut vom Kongreß errichtet wurde. In diesen wenigen Jahren wuchs das Institut aus einem Stab von weniger als 1 Dutzend Mitgliedern auf seine jetzige Größe von über 200 Wissenschaftlern und Technikern mit einem jährlichen État von über 15 Millionen Dollar. In diesen Jahren dehnte sich auch das wichtige Außenprogramm des Institutes, unter dem Gelder für bundesfremde Einrichtungen zur Forschung und Lehre zur Verfügung gestellt werden, von jährlich 200,000 Dollar für 5 Stellen zu einem Fond von über 10 Millionen Dollar aus, der über 400 Forschungsstellen und 300 Lehrstellen an einigen 100 Instituten in allen Gebieten der USA und in einigen anderen Ländern unterstützt.

Wissenschaftliche Durchbrüche, wie die Fluoridierung bei der Kariesprophylaxe können die Tatsache nicht verdecken, daß die Ursache der Erkrankung immer noch nicht bekannt ist. Deshalb werden die vielversprechenden Befunde an Tieren, nach denen Karies eine übertragbare Krankheit bakteriellen Ursprungs ist, energisch weiter verfolgt. Diese Befunde haben eine weitgehende Bedeutung für die Gesundheit der Mundhöhle, weil sie Erscheinungen wie Kariesresistenz und -anfälligkeit erklären, die früher völlig auf genetische Faktoren und Defekte in der Systementwicklung, hervorgerufen durch Ernährungszustand und Ernährungsart, zurückgeführt wurden. Ihre Verwendung für klinische Untersuchungen bedeutet vielleicht die Möglichkeit einer immunologischen oder chemotherapeutischen Verhütung von Karies.

Die biochemischen Untersuchungen des kariostatischen Effektes von Mineralphosphaten im Futter von Tieren haben die Aufmerksamkeit auf das chemische Verhalten der Zahnoberflächen gelenkt. Andere Überlegungen schließen die Substrate der Bakterien der Mundflora und ihre Stoffwechselprodukte ein, die sich in signifikanter Weise in Gegenwart freier Phosphationen verändern können.

Der Fortschritt der biologischen Forschung unterstreicht die Notwendigkeit vermehrter Untersuchungen auf dem Gebiet der periodontalen Erkrankungen, die für den größten Teil der Zahnextraktionen verantwortlich sind. In Anbetracht der immer älter werdenden Bevölkerung werden neue Forschungen über alternde Mundgewebe ermutigt und unterstützt.

Durch seine Geldbewilligungen und Hilfsprogramme fährt das Institut fort, zahnärztlichen Forschungsprojekten wachsende Unterstützung angedeihen zu lassen, besonders auf den weiten Gebieten der periodontalen Erkrankungen, der angeborenen Mundhöhlenanomalien und der Zahnkaries.

Lehrzentren zur Ausbildung gibt es nun in beinahe 30 Instituten in den USA und neue werden in zunehmender Zahl eingerichtet. Aus diesen Zentren wird eine neue Generation zahnärztlicher Wissenschaftler und Lehrer kommen, die für biologische Forschungen ausgebildet sind und die für die Verhütung von Krankheiten in der Mundhöhle arbeiten werden.

Vol.
196

Arch. Oral. Biol., Special Supplement, Vol. 6, pp. 317–329, 1961. Pergamon Press Ltd.

ALTÉRATIONS RÉNALES ET HÉPATIQUES CHEZ DES RATS ALBINOS TRAITÉS PAR MICRODOSES DE NaF

S. Fiorentini et F. Di Blasi

Clinica Odontoiatrica, Università, Roma, Italia

Résumé—Les auteurs afin d'étudier histologiquement les effets des sels de fluor sur le foie et les reins ont administré par voie buccale à 80 rats du NaF à la dose de 0,02 mg % par kg de poids.

Les animaux furent décapités le 180ème jour d'expérimentation.

L'examen histologique des reins révéla chez 45 pour cent des animaux soumis traitement des altérations de l'épithélium tubulaire dont les cellules présentaient des signes de lésions allant jusqu'à la nécrose et, en certains cas, l'occlusion de la lumière tubulaire.

Pour ce qui regarde le foie, les auteurs observèrent chez 35 pour cent des rats soumis au traitement de légères altérations cellulaires tandis que le dessin hépatique était en général bien conservé.

Au total les altérations rénales et hépatiques n'ont pas été importantes; toutefois, même en admettant la correspondance imparfaite de ce qui est observable chez les animaux d'experimentation avec ce qui se passe chez l'homme, les auteurs trouvent qu'un contrôle expérimental d'un remède comme le fluor, fourni à des populations entières à travers la fluoration des eaux potables, doit procurer des données non équivoques, dans le sens que nulle différence doit se remarquer entre les groupes de contrôle et ceux d'expérimentation. Les auteurs, par conséquent, terminent en affirmant la nécessité de contrôles biologiques ultérieurs qui prouveraient la réelle innocuité du fluor aussi dans les doses prophylactiques pour la carie dentaire.

Abstract—The authors, in order to study histologically the effect on the liver and kidneys of salt-fluor, have administred NaF orally to eighty rats, at the dosage of 0.02 mg % per kg of weight. The animals were decapitated on the 180th day of the experiment.

The histological test of the kidneys revealed, in 45 per cent of the treated animals, some alterations at the expense of the tubular epithelium whose cells showed signs of having been affected by the salt-fluor to the point of necrosis, with occlusion in some cases of tubular lumen.

As regards the liver, the authors observed in 35 per cent of the rats submitted to treatment, slight cellular alterations, whereas the shape of the liver appeared generally well preserved.

On the whole the renal alterations were not important; nevertheless, though admitting the not perfect correspondence of what is to be seen in experimental animals with what presumably happens in man, the authors think that the experimental control of a drug like fluor, supplied to a whole population through the fluoration of drinking waters, must furnish unmistakable data in so far as no difference has been noticed between the control and experimental groups. The authors,

therefore, conclude by stating the need of further biological controls demonstrating also the actual harmlessness of fluor in prophylactic doses for decayed teeth.

Zusamenfassung—Die Verfasser haben, um die Wirkungen des Fluorsalzes auf die Leber und die Niere zu studieren, 80 Ratten durch den Mund NaF in Dosen von 0,02 mg.% per Kg Gewicht gegeben. Die Tiere wurden am 180. Tag des Experiments geköpft.

Die histologische Untersuchung offenbarte in 45 Prozent der behandelten Tiere einige Veränderungen des röhrenförmigen Ephiteliums, dessen Zellen Zeichen von Leiden bis Nekrose zeigten, mit Verschliessung, in einigen Fällen, des röhrenförmigen Lumens.

Was die Leber anbetrifft bemerkten die Verfasser in 35 Prozent der Ratten, die der Behandlung unterworfen waren, leichte Zellenveränderungen, während das Leberbild gewöhnlich gut erhalten blieb.

Im allgemeinen sind die Nieren -und Leberveränderungen nicht bedeutend; dennoch, obwohl durch die Beobachtungen kein vollkommener Zusammenhang zwischen den zum Experiment gebrauchten Tieren und Menschen zu bemerken ist, meinen die Verfasser, dass die Experimentalkontrolle, von einem Medikament wie Fluor unzweideutige Daten ergeben muss, in dem Sinne, dass es keinen Unterschied zwischen Kontroll-Gruppen und Experiment-Gruppen geben darf.

Und so schliessen die Verfasser damit ab die Notwendigkeit von weiteren biologische Kontrollen bestätigend, um die wirkliche Unschädlichkeit des Fluors, auch im Gebrauch der prophylaktischen Dosen für Zahnfäule, festzustellen.

LA FLUORATION artificielle de l'eau potable, largement réalisée aux États Unis d'Amérique afin d'entreprendre une prophylaxie adéquate de la carie dentaire, a subi un arrêt pendant ces dernières années.

En effet, cette méthode d'administrer le fluor, malgré un dosage exact dans l'eau, ne permet pas de calculer l'absorption réelle de chaque individu; le besoin individuel d'eau peut varier selon la température ambiante, l'âge et les divers états de santé. (BENAGIANO, 1958). En outre, tous les produits du sol mêmes ceux des régions non endémiques, contiennent des quantités plus ou moins élevées de fluor dont la consommation fournit en moyenne 1 mg par jour de cet halogène (MINKH, 1954). De plus le fluor ajouté artificiellement peut être plus nuisible que celui qui se trouve dans la nature.

A cause de ces considérations, dans le cours de peu d'années, 477 villes des États Unis ont refusé de réaliser la fluoration, ou l'ont interrompue après l'avoir entreprise (ROBINSON, 1955). Les données des auteurs qui soutiennent l'innocuité d'un tel système de prophylaxie, ne pourraient être plus unilatérales.

Tous les auteurs qui ont soutenu la fluoration de l'eau potable, sont convaincus que l'absorption d'eau contenant 1 p.p.m. de fluor est absolument à l'abri de tout dommage. Il est étonnant que malgré les nombreuses régions des États Unis où la fluoration artificielle de l'eau potable a été réalisée il y a plus de dix ans, on n'ait pas encore fait une étude approfondie des larges couches de la population, de tout

âge, afin d'en contrôler, moyennant des données cliniques et biologiques, l'état des différents organes plus sensibles à l'intoxication par le fluor, et d'en relever non seulement les dommages qui pourraient se manifester éventuellement, mais aussi ceux plus larvés et latents.

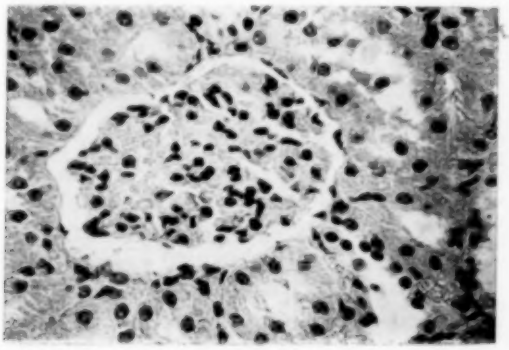


FIG. 1. Rein. Le protoplasme de l'épithélium tubulaire paraît granuleux (AT1).

Le Dr. DYMOND, Ministre de la Santé de l'Ontario, nous fournit la meilleure synthèse de ce qu'on peut dire aujourd'hui à propos de la fluoration: "Quand je prescris un remède à mes patients, j'en prescris une quantité établie". Puisqu'il

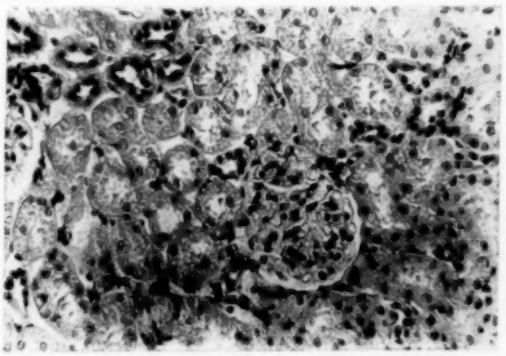


FIG. 2. Rein. Légère ischémie du glomérule, granulosité dans le cytoplasme des cellules épithéliales (AT1).

est impossible d'administrer une quantité précise de F en l'introduisant dans l'eau potable, il est plus juste de penser que la prophylaxie anti-carie future devra se développer sur la base de l'administration individuelle et contrôlée du fluor.

L'École de Rome a depuis longtemps entrevu les dangers d'un système de prophylaxie anti-carie semblable. BENAGIANO et ses collègues ont fait une série

de recherches cliniques et expérimentales dans le but de constater si la fluoration de l'eau potable peut être par elle-même "une cause de maladie". Pendant ces expériences on a pu démontrer l'apparition d'altérations histologiques de la thyroide des animaux intoxiqués par le NaF (BENAGIANO et FIORENTINI, 1955) et des

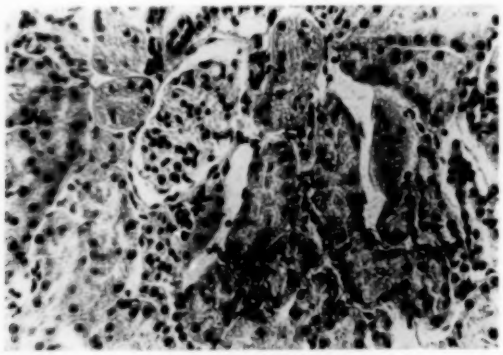


FIG. 3. Rein. Les glomérules sont ischémiques (AG1) tandis que dans l'épithélium tubulaire on observe des zones de légère dégénération vacuolaire (AT2).

troubles fonctionnels de la thyroide, ainsi que de l'altération de la formule sanguine chez les sujets qui vivaient dans les régions où le fluor était présent à dose optimale (BENAGIANO, DE SIMONE et COLASANTI, 1959).

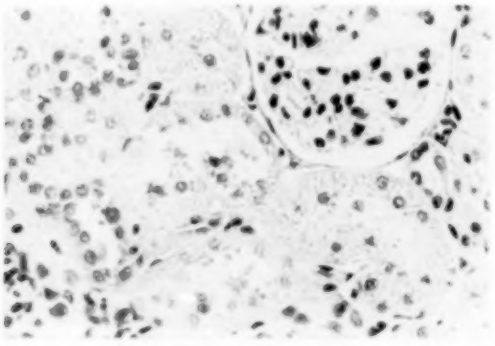


FIG. 4. Rein. Altérations tubulaires de IIIème degré; nécrose des cellules épithéliales (AT3).

Le but de ce travail est d'établir si, chez les animaux soumis à un traitement de F à bas dosage, et tels qu'ils sont normalement employés pour la fluoration artificielle de l'eau potable, apparaissent effectivement des altérations histologiques du foie et des reins.

L'action sur le foie et les reins des différentes doses de fluor administrées pendant de longues périodes, n'a pas été étudiée suffisamment.

Vol. 6
1961

HODGE (1956) après avoir administré des doses de F radioactif à des rats a étudié la concentration sélective du fluor dans les divers organes; 90 min après l'apport de F radioactif, la quantité plus élevée de F fut trouvée dans les reins (34 075 counts par minute) et dans le foie (9 500 counts par minute).

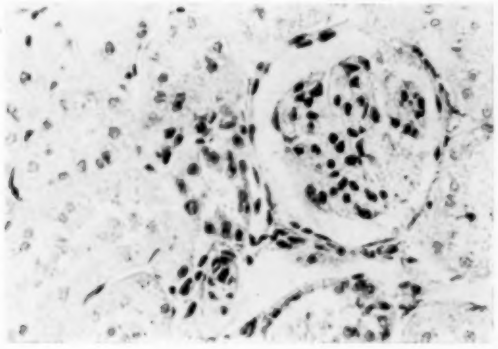


FIG. 5. Rein. Altération à charge des anses glomérulaires (AG2) et nécrose des cellules épithéliales qui ferment la lumière des tubes urinaires (AT3).

L'élimination du fluor par les urines peut atteindre des valeurs très élevées. AGATE (1949) et ses collaborateurs trouvèrent que chez des sujets exposés, l'élimination de F par les reins variait de mg 0,5 jusqu'à mg 23, 3 pendant les 24 heures.

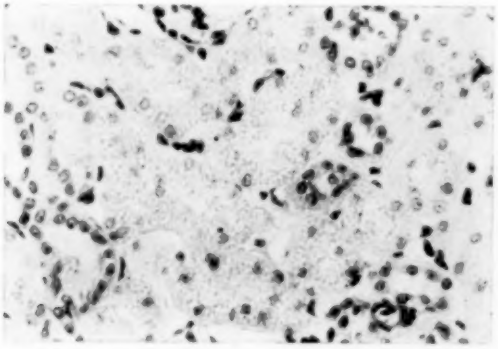


FIG. 6. Rein. Altérations nécrotiques de l'épithélium tubulaire (AT3).

BOND et MURRAY (1952) provoquèrent des cas de fluoroses chroniques chez les rats soumis à des doses massives de fluor; les doses oscillaient de 4 à 15 mg pro die. L'examen histologique des reins révéla des lésions diffuses à caractère dégénérescent des glomérules et plus spécialement de l'épithélium tubulaire.

RAMSEYER (1957) et ses collaborateurs étudièrent les effets des bas dosages de F, administrés dans l'eau de boisson, à un total de 456 rats albinos, de la naissance jusqu'au vieil âge. Les animaux furent partagés en quatre groupes; aux trois premiers on administra du fluor à raison de 1,5 p.p.m.; le quatrième fut gardé pour

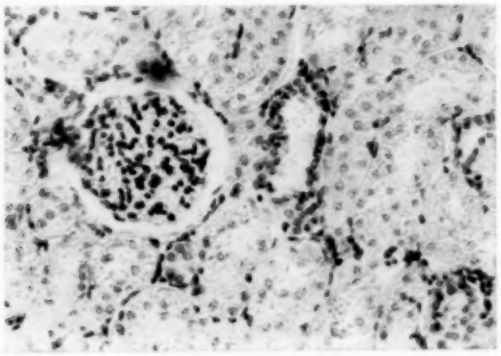


FIG. 7. Rein. Glomérules ischémiques (AG1) graves altérations tubulaires (AT3).

le contrôle. Les auteurs remarquèrent l'hypertrophie et l'hyperplasie des tubes urinaires chez les rats qui buvaient l'eau fluorée, tandis que l'examen histologique des rats de contrôle ne révéla aucune altération; la gravité des données pathologiques augmentait proportionnellement au pourcentage du NaF dans l'eau.

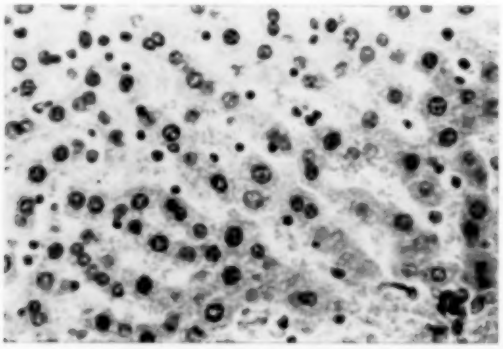


FIG. 8. Foie. Les cellules hépatiques présentent une augmentation de la granulosité du protoplasme cellulaire (AC1).

PINDBORG (1957) administra à 226 rats blancs une diète contenant 0,058 de NaF pendant une période de 3 à 36 jours. Les modifications rénales parurent régulièrement après 21–28 jours du traitement. La fréquence des lésions fut plus élevée chez les rats de 1 mois que chez ceux de 3 mois. Les modifications rénales

consistaient avant tout en la dilatation des anses de Henle dans la partie juxtacorticale de la substance médullaire, immédiatement suivie par l'applaitissement de l'épithelium dans les tubes urinaires contournés de la matière corticale et dilatation des tubes urinaires. L'étude des publications à ce sujet nous porterait donc

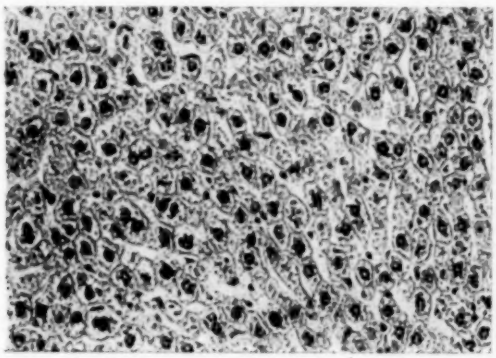


FIG. 9. Foie. L'aspect typique du dessin hépatique est conservé tout en paraissant notablement désorganisé (AD2) signes graves de souffrance cellulaire (AC2).

à croire que le F provoque de sensibles et évidentes altérations rénales; toutefois il nous faut reconnaître que, à part les expérimentations de RAMSEYER, les doses de fluor administrées expérimentalement étaient le plus souvent très élevées; à notre avis il est moins intéressant d'établir la toxicité générale du fluor que de savoir si

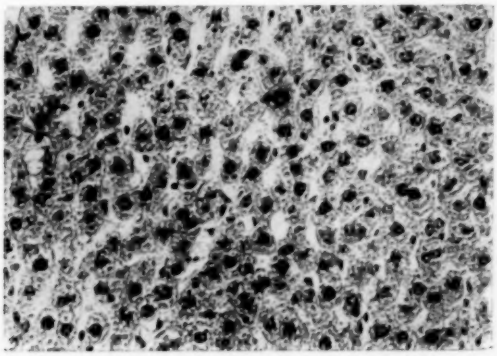


FIG. 10. Foie. Le dessin hépatique est altéré (AD1) avec troubles aussi à charge des cellules parenchymales.

le F administré en quantité suffisante pour assurer une prophylaxie anti-carie adéquate est plus ou moins toxique.

Une étude expérimentale établie selon les microdoses suivantes, nous a semblé particulièrement opportune.

MATÉRIEL ET MÉTHODE

Le traitement a été administré à 80 rats du poids initial de 50–70 g; les animaux furent partagés en quatre groupes marqués des lettres A,B,C,D.

Le groupe A comprenait 40 rats et le groupe D, 20 animaux. On garda le groupe D pour le contrôle; les autres trois groupes furent traités avec trois dosages

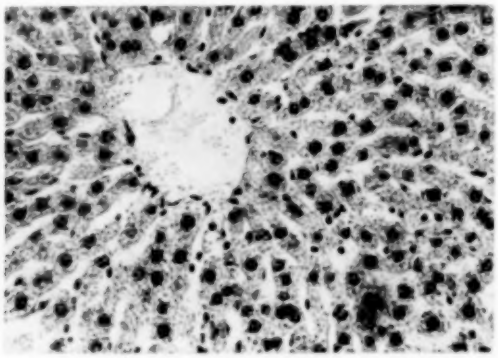


FIG. 11. Foie. Les cellules hépatiques sont gravement altérées (AC2).

différents de NaF. Les doses de NaF administrées journalièrement par voie buccale au moyen de micropipettes furent les suivantes:

Au groupe A: 0,02 mg/kg — dose prophylactique
groupe B: 0,2 mg/kg — dose insuffisante pour déterminer le quatrième degré de la fluorose endémique
groupe C: 2 mg/kg — dose toxique.

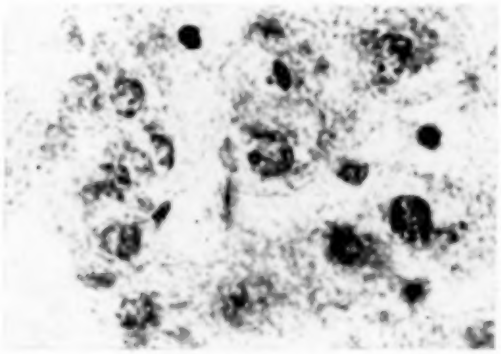


FIG. 12. Foie. Graves altérations de la cellule hépatique (AC2).

Les animaux furent sacrifiés après 180 jours d'expérimentation.

Puis on préleva le foie et les reins pour les fixer, enfermés dans paraffine, coupés par tranches et colorés avec hématoxiline et éosine.

Nous avons voulu soumettre au traitement quelques groupes d'animaux au moyen de doses comparables à celles de la fluorose endémique (groupe B) et à celles encore plus toxiques (groupe C), malgré que dans les publications à ce sujet, se trouve la description des cas toxiques dus aux doses élevées de fluor, surtout pour ce qui concerne les reins. Pour notre part nous avons pensé que dans l'éventualité

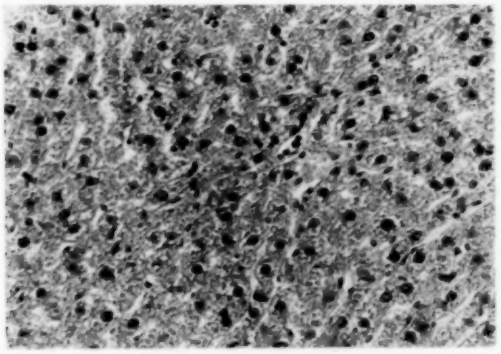


FIG. 13. Foie. Désorganisation du dessin hépatique (AD2) avec signes de souffrance cellulaire (AC2).

de relever des altérations rénales et hépatiques au moyen de doses prophylactiques il aurait été plus aisé de donner un avis sur ces altérations, toutes légères qu'elles soient si nous pouvions les comparer non seulement avec celles des animaux de contrôle, mais avec celles aussi des animaux bien plus intoxiqués et entretenus dans le même état expérimental.

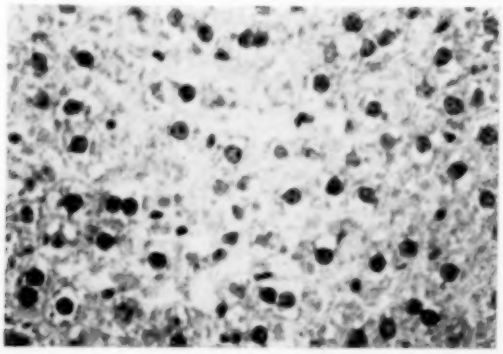


FIG. 14. Foie. Cellules hépatiques en grave état de souffrance (AC3).

Pouvant disposer d'un certain nombre d'animaux nous avons évalué les altérations rénales et hépatiques d'après un criterium quantitatif et qualitatif; il est en effet évident qu'une donnée pathologique qui se présente parfois aussi chez les

animaux de contrôle, acquiert une signification positive si on l'observe non seulement avec plus d'intensité, mais également avec plus de fréquence chez les animaux soumis au traitement. La statistique nous permet ainsi d'obvier aux erreurs d'évaluation dérivées de la pathologie spontanée des rats. Il va sans dire que cette évaluation est valable seulement dans les cas où les animaux de contrôle présentent des signes de pathologie spontanée de peu d'importance ou de toute façon étrangers au fluor et, à part celà, dont les caractéristiques morphologiques ne présentent aucune difficulté diagnostique particulière. C'est en suivant cette idée que nous avons écarté complétement ces groupes d'animaux qui présentaient des signes pathologiques plus évidents; ce qui fait que, tandis que dans ce travail nous rapportons les données fournies par 80 rats, l'ensemble de nos expérimentations nous a amenés à étudier 250 animaux environ.

Évaluations des altérations hépatiques

Dans cet organe nous avons distingué: Les altérations cellulaires (AC) et les altérations du dessin (AD).

Les altérations cellulaires (AC) ont été classifiées selon leur degré d'intensité en
Modestes (AC1)
Graves (AC2)
Nécrotiques (AC3)

Les altérations cellulaires peuvent intéresser la substance nucléaire, par la pycnose ou par la faible réaction au couleurs des tissus et le protoplasme par un accroissement de granulosité et déperdition des limites cellulaires.

Nous avons distingué les altérations du dessin (AD) caractéristiques de la glande en

Modestes (AD1) lorsqu'on estimait une altération à peine initiale du dessin
Graves (AD2) lorsque la désorganisation du dessin était d'une importance accentuée, mais l'aspect typique du dessin hépatique était encore reconnaissable
Profondes (AD3) dans les cas où le dessin de la glande était absolument méconnaissable.

La dernière donnée pathologique que nous avons considérée est l'infiltration cellulaire.

La reconnaissance de cette donnée a été pour nous un élément précieux en tant que là où nous l'avons observée, elle nous a servi à exclure l'influence du fluor, pour le moins comme cause principale de l'altération hépatique rencontrée; le F en effet étant un toxique cellulaire, n'occasionne pas d'infiltrations dans le tissu d'appui.

Évaluation des altérations rénales

Nous avons distingué les altérations rénales en altérations tubulaires (AT) et altérations glomérulaires (AG).

Les altérations tubulaires (AT) ont été classées, toujours pour faciliter notre étude, bien entendu, en trois degrés:

I degré (AT1) granulosité du protoplasme cellulaire
II degré (AT2) dégénération vacuolaire
III degré (AT3) nécrose de la cellule avec éventuelle oblitération de la lumière du tube.

Nous avons distingué les altérations glomérulaires (AG) en:

I degré (AG1) ischémie du glomérule
II degré (AG2) altérations des anses qui peuvent se présenter oblitérées ou contournées
III degré (AG3) quand au Ier et IIème degré s'associent les altérations de la capsule de BOWNMAN qui présente une membrane pluristratifiée.

Quant à l'infiltration cellulaire, voyez ce qui a été dit pour le foie.

Vol. 6
1961

Données histologiques

Les altérations histologiques plus significatives des reins et du foie des rats soumis au traitement de microdoses de NaF ont été rapportées dans les microphotographies. Dans le tableau qui suit nous donnons un aperçu aussi complet que possible de la fréquence de chaque altération rencontrée.

CONSIDÉRATIONS ET CONCLUSIONS

Dans le tableau 1 nous avons synthétisé la fréquence des altérations histopathologiques relevées dans le foie et reins des animaux de contrôle, et de ceux auxquels a été administré le NaF en dose prophylactique; de cette façon le tableau nous donne une description synthétique des altérations rencontrées, soit chez les animaux d'expérimentation, soit chez les animaux de contrôle.

TABLEAU 1. POURCENTAGE DES RATS (GROUPES A ET D) PRÉSENTÉ DES ALTÉRATIONS RÉNALES ET HÉPATIQUES SELON DIFFÉRENTS DEGRÉS D'INTENSITÉ

	AT0	AT1	AT2	AT3	AG0	AG1	AG2	AG3
A	55%	17,5%	15%	12,5%	65%	20%	7,5%	7,5%
D (Contrôles)	85%	15%	—	—	85%	15%	—	—

	AC0	AC1	AC2	AC3	AD0	AD1	AD2	AD3
A	65%	10%	15%	10%	75%	12,5%	10%	2,5%
D (Contrôles)	90%	—	10%	—	80%	10%	10%	—

Quant au rein, tandis qu'il n'y a pas de différence particulière entre les altérations glomérulaires rencontrées chez les deux groupes d'animaux, pour ce qui regarde les altérations tubulaires nous avons pu relever une différence sensible entre les

deux groupes: en effet les altérations tubulaires du IIIème degré ont paru seulement chez les animaux ayant subi un traitement de F avec une fréquence du 12,5%: toujours chez les animaux soumis au traitement, les altérations tubulaires du IIème degré ont paru avec une fréquence de 15% et celles du Ier degrée, de 17,5%; chez les rats de contrôle les seules altérations tubulaires du Ier degré ont paru avec une fréquence de 15%.

A l'égard du foie le procédé a été analogue; les altérations de la cellule hépatique ont été beaucoup plus sensibles chez les rats soumis au traitement; en effet les altérations cellulaires nécrotiques (AC3) ont donné une fréquence de 10%, les altérations cellulaires graves (AC2) de 15% et celles modestes (AC1) de 10%; par contre chez les animaux de contrôle, pas d'altérations du Ier et IIIème degré tandis que celles de IIème degré n'ont paru que chez 10% des animaux.

Une différence appréciable des altérations du dessin de la glande entre les animaux soumis au traitement et ceux de contrôle, n'a pas été remarquée. Il nous faut encore préciser que tous les rats qui à l'examen histologique démontraient des dérangements hépatiques, présentaient des altérations rénales; tandis que chez quelques rats présentant des altérations rénales, le foie ne s'était pas ressenti du traitement. Ceci pourrait signifier que le fluor agit plus activement sur le rein, peut-être parce que cet organe, en sa qualité d'émonctoire, est en contact avec la quasi totalité du fluor introduit dans l'organisme n'importe comment.

Nous avons en outre pu constater que chez les groupes soumis aux doses plus élevées de fluorure de Na, les altérations rénales et hépatiques avaient un degré plus élevé, mais pas proportionné à la dose administrée.

Nous avons considéré significatives à l'égard de l'intoxication par le fluor, les altérations des tubes urinaires et celles de la cellule hépatique, altérations qui soit pour l'un, soit pour l'autre organe sont pathognomoniques d'un facteur toxique, qui dans notre cas doit être individualisé dans le F qui est un poison protoplasmique.

En somme les altérations rencontrées, soit rénales soit hépatiques n'ont pas été de grande importance; toutefois, même en admettant la concordance imparfaite de ce qui est observable chez les animaux d'expérimentation avec ce qui censément se passe chez l'homme, nous pensons que le contrôle expérimental d'un remède fourni à toute une population, doit procurer des données non équivoques, dans le sens que nulle différence doit se remarquer entre les groupes de contrôle et ceux d'expérimentation.

A la suite de nos recherches nous pouvons affirmer que les données fournies par les animaux d'expérimentation ne sont pas égales en fréquence et en intensité avec celles des animaux de contrôle, du moment qu'on a rencontré chez les premiers des altérations de peu d'importance, mais de nature pathologique certaine, qui chez les animaux de contrôle ne paraissent absolument pas.

Il faut à présent nous demander quelle doit être l'opinion sur la fluoration de l'eau potable d'après nos données.

Les observations cliniques établies par BENAGIANO *et al.* (1959) dans certaines zones ayant des doses optima de F, concernant la fonction thyroïdienne et la crase sanguine, comparées avec nos observations expérimentales, donnent les unes et les autres du crédit à la thèse de notre école, qui condamne la fluoration de l'eau potable comme une application prophylactique qui tout en servant à la santé dentaire de couches limitées de la population, peut provoquer des dommages irréversibles à certains organes d'importance vitale, notamment chez des individus adultes, atteints des maladies les plus variées, ou qui, par leur état de santé, sont plus facilement exposés à l'action des microdoses de F.

RÉFÉRENCES

AGATE, J. N. 1949. *Industrial Fluorosis*. Brit. Med. Res. Council Memo No. 22.

BENAGIANO, A. 1958. *Ann. Stomat.* **7**, 3.

BENAGIANO, A. et FIORENTINI, S. 1955. *Ann. Stomat.* **4**, 3.

BENAGIANO, A., DE SIMONE, G. et COLASANTI, A. 1959. *Ann. Stomat.* **8**, 1.

BOND, A. M. et MURRAY, M. M. 1952. *Brit. J. exp. Path.* **33**, 168.

HODGE, H. 1956. *J. Amer. med. Ass.* **52**, 307.

MINKH, A. A. 1954. *Stomatology, Kbh.* **2**, 5.

PINDBORG, J. J. 1957. *Acta Pharm. tox., Kbh.* **13**, 36.

RAMSEYER, W. F. 1957. *Gerontology* **12**, 14.

ROBINSON, MRS A. R. 1955. List of cities defeating, rejecting or that have thrown out fluoridation of water supplies. Compiled by Mrs. A. R. ROBINSON, 5217 E 43rd Street, Seattle 5, Washington, U.S.A.

Vol. 6
1961